CHEMISTRY OF NATURAL PRODUCTS AND RELATED COMPUNDS

DISSERTATION SUBMITTED
IN PARTIAL FULFILMENT FOR THE DEGREE
OF

Master of Philosophy

IN

CHEMISTRY

ALIGARH MUSLIM UNIVERSITY, ALIGARH

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INSTITUTE OF HISTORY OF MEDICINE
AND MEDICAL RESEARCH
(FACULTY OF SCIENCE, JAMIA HAMDARD)
NEW DELHI, (INDIA)

1990

D.

This is to certify that the work contained in this dissertation entitled "Chemistry of Natural Products [illegible] compounds", submitted in partial fulfilment of the requirement for the degree of Master of Philosophy of Aligarh Muslim University, Aligarh, has been carried out by Mr. Muhammad Asif, under our direct supervision and guidance.

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ACKNOWLEDGEMENT

I feel deeply obliged to express my profound gratitude to my esteemed guide Prof. Dr.M.S.Y.Khan, Head, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi. I am also grateful to Prof. M. Ilyas for his kind help and interest during the course of these studies.

I am thankful to Alhaj Janab Hakeem Abdul Hameed Sahib Chancellor of Jamia Hamdard for providing me research facilities.

I am also grateful to Mr. Abdul Mueed, Chief Mutawalli Hamdard National Foundation for his kind interest and encouragement.

I am also indebted to Janab Professor Hm.Jamil Ahmed, Dean Faculty of Medicine for all the encouragement which he gave me during these research studies.

I am highly indebted to Mr. Kalim Javed and Dr.Monammad Hasnain Khan, my senior colleagues with whose constant and sincere help, I could carryout my research work. I am also thankful to Dr. Sacod Husain.

With deep regards I acknowledge the inspiration which received from my father, Alhaj Janab Muhammad Yusuf Saheb. His deep affection has always been an insiring force in me.

My thanks are also due to Mr. Ahsanul Haq & Mr.Rehmanul Haque assisting me in the day to day research work.

(MUHAMMAD [illegible]

The work described in this dissertation was carried out in the department of Medicinal Chemistry of the Institute of History of Medicine and [illegible] search (Jamia Hamdard) New Delhi under direct supervision [illegible] idance of Prof. M.S.Y. Khan Head Department of [illegible] istry, Faculty of Pharmacy, Jamia Hamdard.

I am highly grateful to Prof. M.S.Y. Khan for his valuable guidance and constant help and encouragement extended throughout these studies.

(MUHAMMAD ASIF)

C O N T E N T S

I N T R O D U C T I O N

INTRODUCTION

The use of plants or plant extracts for medicinal purposes has been going on for thousands of years and herbalism and folk medicines, both ancient and modern, have been the source of much useful therapy. The traditional system has provided a large number of medicinal plants of therapeutic action.[1,2] Unani tibb and Ayurveda are the two most common traditional systems of medicine in India. It is estimated that about 60-70% population of India gets their treatment from traditional medicine[3].

A traditional novel approach for the development of new active medicinal agents is the exploration of nature. The flora and fauna has been successfully exploited to the benefit of mankind since the dawn of history. A survey done by WHO shows that about 80% of world population depends chiefly on traditional medicines.[4] Plant derived drugs get an important place in developed countries too. Plant extracts or active principles prepared from higher plants are about 1/4th part of all the prescriptions dispensed from community pharmacies in U.S.A.[5,6]

Its impact on drug development is two folds. It often provides potent drugs or gives useful leads which require further exploitation and the leads can be suitably manipulated to synthesise biologically active compounds. The medicinal chemist plans modifications in the light of various physicochemical properties that

enhance the availability and interaction of drugs with receptor site. During the last 100 years many active ingredients present in herbal prescriptions have been isolated and introduced into 'modern' medicine. There are atleast 199 different chemical substances used in USA as important drugs which have been derived from plants as revealed by Fransworth et al.[4]

Below are being mentioned some important biological actions of naturally occurring triterpenoids.

Triterpenoids have been extensively investigated for their biological actions which revealed a broad spectrum of pharmacological and physiological activities[7] such as antibacterial, antifungal, antiinflammatory, antineoplastic, spermicidal, diuretic, antidiabetic, central nervous sytem acting, metabolite displacing, ascaricidal, molluscicidal and as antifeedants. Some of the triterpenoids are being used clinically as standard drugs.

It has been found that with increasing polarity of oleanene series which means that an increase in the number of hydroxyl groups in the molecule[9] the antiinflammatory activity increases. Against carrageenan-induced oedema and formaldehyde induced arthrities in rats, the triterpenoids of the oleanene and ursene series have been found to be active.[8] Oleanolic acid-3-B-glucoside has been found to be significantly active in the proliferative and exudative phase of inflammation in rats[10]. Gastric ulceration induced in rats has significantly lowered by giving taraxerol, lupeol and ursolic acid[11]. An appreciable antiinfla-

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mmatory activity was exhibitted by glycyrrhetic acid[12] from Glycyrrliza glabra, boswallic acid[13] from Boswellia serrata and bassic acid[14] from Bumelia sartorum.

Antineoplastic activity has been exhibitted by Tinogenone[15-17] cytotoxic[18-26] and anticancer[18-21] activities are present in cucurbitacins. Maytenfolic acid,[27] zeorin and missourin[28] also possessantineoplastic action.

The highest fungicidal activity has been reported of the pentacyclic triterpene glucosides of oleanolic acid and hederagenin with a free carboxylic group at C-28 or C-27[31]. Maytenonic acid[29], oleanolic acid and ursolic acid[30] also have been found to have antibacterial activity. Antibiotic activity has been found to be present in the two tetracyclic triterpenoids, fusidic acid and helvolic acid.

2,3-diacetate of 2α, 3β, 20β, urs-12-ene-24β,28 dioic - acid, a new triterpenic acid, isolated from Corchorus depressus produced a non-narcotic type of analgesia against acetic acid induced writhing and electrical noxious stimuli in rats. Anti-pyretic effect has also been shown by it[32]. Significant analgesic activity against thermal stimulus in rats was shown by oleanolic acid 3β-D-glucoside[10].

A good hypoglycemic activity was found in tormentic[34] acid and bassic acid[14].

In experimental animals diuretic activity was displayed by friedelin, 16α-hydroxy-3-ketoisomultiflorene and 3β-hydroxy 16-ketoisomultiflorene[33].

It has also been found that cholesterol absorption in experimental animals is lowered by triterpenoids.[35,36]

The two triterpenes tricoumaric acid A and B isolated from Tricoumaria roka have been found to have antifeedant activity against Spodoptera litura, southern army worm and North America pest insects[42,43]. Toonacilin and 6-acetoxy toonacilin isolated from toonaciliata exhibitted antifeeding activity against Epilachna vervestis[44]. From Jacquinea pungens, the jacquinonic acid has been found to be antrepellent triterpenoid.[45]

An ascaricidal triterpene, chuanliansu has been reported from the bark of Melia toosendan[37]. This compound has low toxicity and is therefore preferred by pedestricians[38].

Niamocinolide and isonimocinolide isolated from the fresh leaves of Azadiracnta indica act as insect growth regulators against house flies and mosquitoes[41]. Azadirachtin and deacetylazadirachtinol are effective antifeedants.[39,40]

In the present investigations we have chemically examined the two important medicinal plants namely :

(i) Ichnocarpus frutescens (R.Br.),

(ii) Corchorus acutangulus,

for their chemical constituents. These herbs were found to have triterpenic acids. The details of experimental work and structure elucidation of these compounds have been given in the discussion and experimental parts of this thesis. A review article on triterpenes has also been embodied in the thesis giving details of different types of skeletons present in this series of compounds and the methods used to establish the structures of triterpenoids.

THEORETICAL

TRITERPENOIDS

The term 'Triterpenoid' refers to a very large group of naturally occurring substances, with a carbon skeleton based on 6 isoprene units which are derived biosynthetically from an acyclic C_{30} hydrocarbon squalene. There are probably more than 500 natural triterpenoids of established structure. Although the isolation of many important triterpenoids dates back to the last century, the first correct structure was not designed until 1937 when Haworth[46], Ruzicka et al.[47] and Ames et al.[48] correctly formulated parent substances α-amyrin, β-amyrin and lupeol respectively.

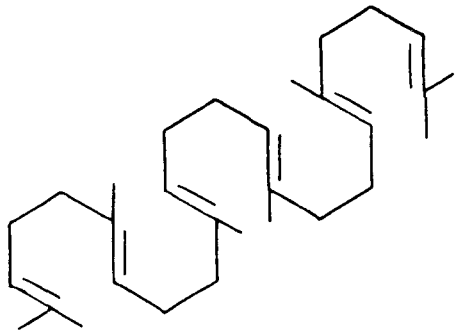
Triterpenoids may be cyclic or acyclic, broadly classified on that basis. They have relatively complex cyclic structures, mostly being either alcohols, aldehydes or carboxylic acids. They are colourless crystalline, often high melting, optically active substances which are generally difficult to characterise because of their lack of chemical reactivity.

Many triterpenes are known in plants and new one are being discovered and characterised.[49] In plants, they are found in resins, barks of trees, latex (Euphorbia, Havea etc.) and in plant saps in free state and as esters or glycosides (saponin). A few have been found in animal sources e.g. in liver oil of certain fish, especially of shark family (e.g. lanosterol).

The true pentacyclic triterpenes, α and β-amyrin, their derived acids and related compounds occur specially in waxy coating of leaves and fruits such as apples, pears and they may have protective function in repelling insects and microbial attacks. Limonoids and

quassinoids series of pentacyclic triterpenes, notable for their bitterness occur principally in Rutaceae, Meliaceae, and Simaroubaceae[50]. Another group of triterpenes are cucurbitacins, confined mainly to the seed of various cucurbitaceae but recently detected also in the crucifere, in Iberis.[51]

The broad classification of triterpenoids is based on acyclic and cyclic triterpenoids. An example of acyclic triterpenes is squalene (I), which is considered to be the precursor of the cyclic type.

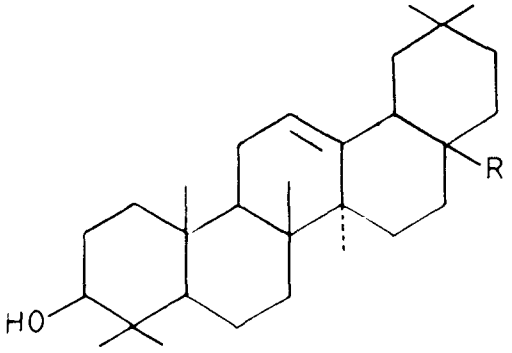


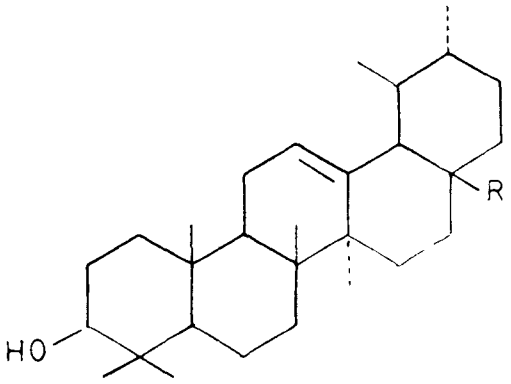
(I)

On the basis of rings present, two systems commonly met with are the tetracyclic and pentacyclic compounds. Lanosterol and other compounds such as euphol, euphorbol and elmi acids found in various resins belong to the former group[52].

The pentacyclic triterpenes were earlier subdivided into three main groups, namely β -amyrin (II R=CH_3). $\mathfrak{L}$ -amyrin(III R=CH_3) and lupeol system (IV R=CH_3). The parent saturated hydrocarbon of these are called oleanane, ursane and lupane derived from ole-

anolic acid, ursolic acid and leupeol which are typical examples of these systems.



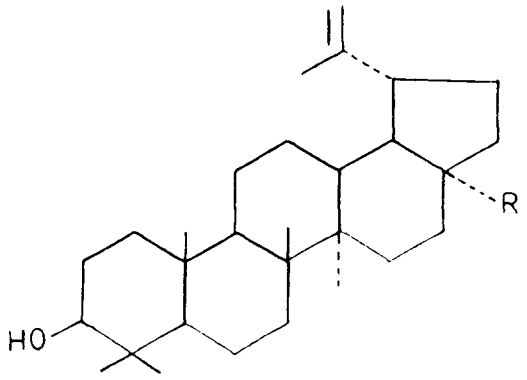


(II) R=CH_3' β amyrin

R= COOH, Oleanolic acid

(III) R=CH_3, αamyrin.

R = COOH, Ursolic acid



(IV) R = CH_3, Lupeol

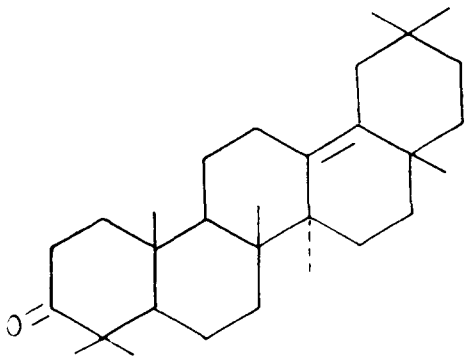
R = CH_2OH, Betulin

Since the major review of the chemistry of pentacyclic triter-

penes by White[53] published in the year 1956 and subsequent review by Boiteau et al[54], there has been a continuous activity in this field and many new compounds have been isolated and their structures are established. As early as upto 1950 the aim of chemists working with triterpenes was to relate them to the limited number of widely occurring parent ($C_{30}H_{50}O$) alcohol then known e.g. oleanolic acid was related to β-amyrin, ursolic acid to α-amyrin and betulin to lupeol.

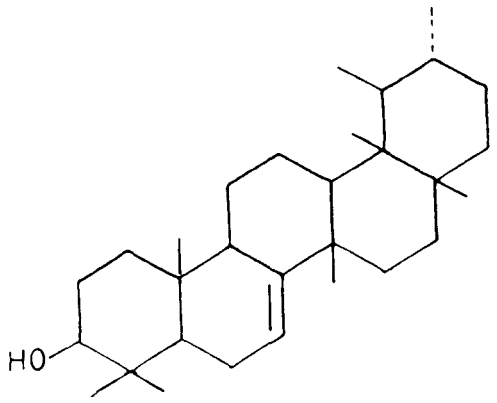
The structures of these parent alcohols were determined by very tedious methods involving degradation of these compounds. However, at this stage very little was known about the stereo-chemistry of the triterpenes.

Jones et al.[55,56] in the year 1949 took a big step forward by establishing a relationship between lupeol (IV R=CH_3) and β-amyrin (II R=CH_3), the corresponding ketones being both converted by acid to a mixture of δ-amyrenone (V) (Olean-13(18)-en-3one) and 18-iso-olean-12-en-3one[57] and in this way whatever structural features were established for β-amyrin these could be applied to lupeol also.



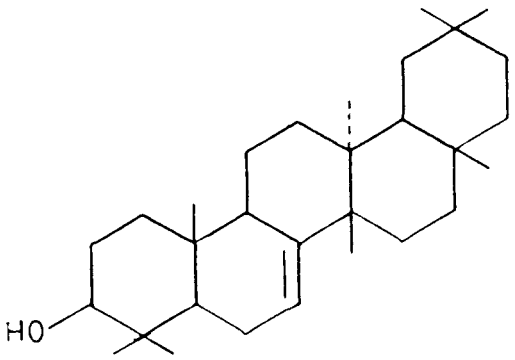
(V) δ-Amyrenone

Similarly the acetate of bauerenol (VI) was isomerised by boiling with hydrochloric acid to a mixture of £-amyrin acetate and the 13(18) isomer-urs-13(18)-en-3 β -yl acetate, and the acetyl derivative of oleanane analogue of bauerenol -multiflorenol (VII) was isomerised with chloroformic hydrogen chloride to β -amyrin acetate.



(VI)

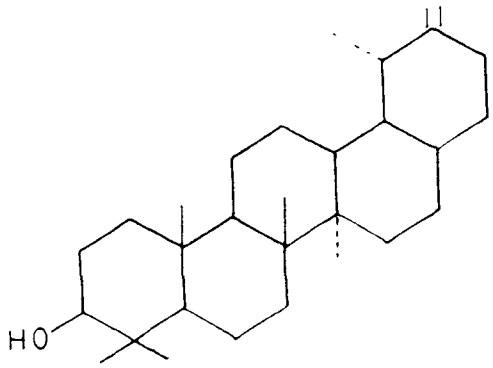
Bauerenol



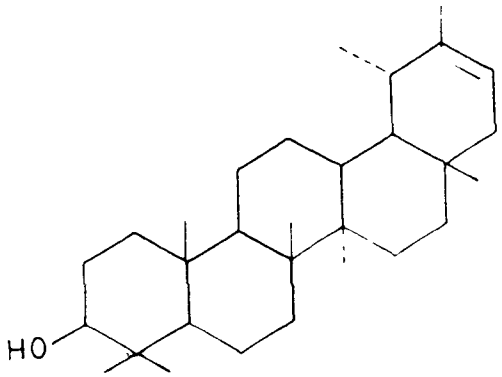
(VII)

Multiflorenol

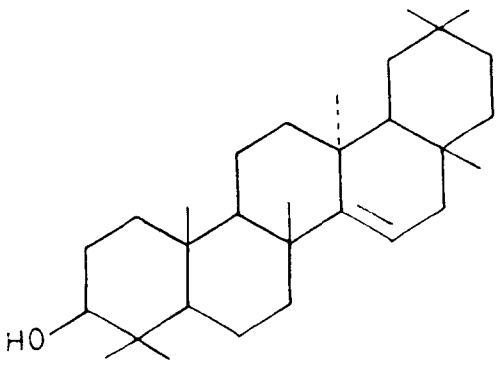
Other, transformations of some suitable derivatives of taraxasterol (VIII), ψ-taraxasterol (IX), taraxerol (X), glutinol (XII) and friedelin (XI) to olean-13 (18)-en-3one or the corresponding hydrocarbon have also been reported in the literature[58-64].



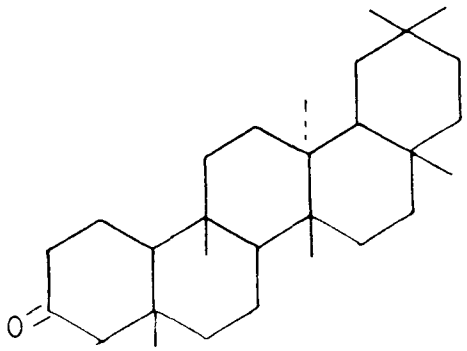
(VIII)
Taraxasterol



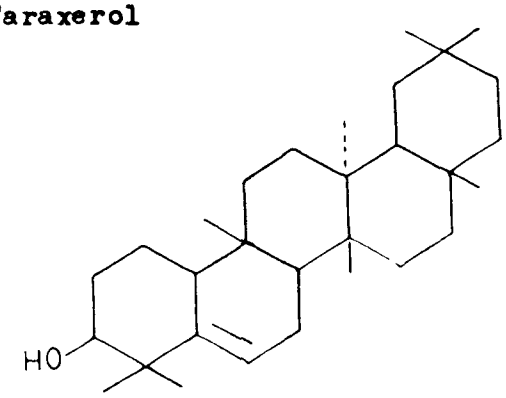
(IX)
ψ-taraxasterol



(X)
Taraxerol



(XI)
Friedelin



(XII)
Glutinol

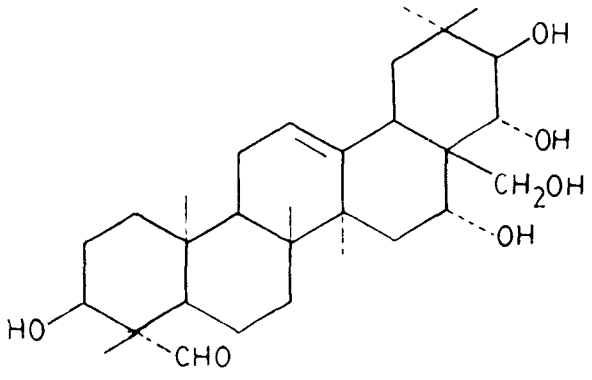
Hydroxylation pattern in triterpenes

In triterpenes as in steroids 3-hydroxyl group is ubiquitous

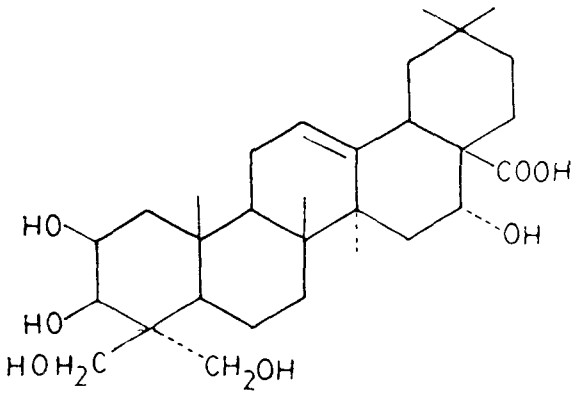
and often noticed as a point of attachement to the sugar residue in saponins[65,66].

The polyhydroxy β-amyrins possess hydroxyls in other positions also, in addition to the 3-positions. The frequency of hydroxylation is in the following order 16,22,21,19,6,7 and 15 among ring methylene positions while the hydroxylation of the angular methyl group is in order of 23,28,30,27 and 29 positions. These hydroxyls occur in numerous combinations also such as di, tri, tetra,penta, and hexa-hydroxy β amyrins.

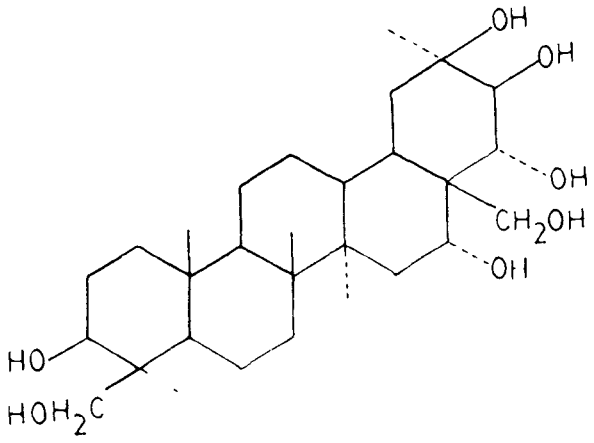
By a study of the chemistry of triterpenes an impressive fact emerges out that β -amyrin type of compounds occur widely in nature as alcohols or hydroxy carboxylic acids. Hydroxylation of olean-12-ene skeleton is so extensive that pentahydroxy and hexahydroxy β -amyrin also occur in nature. Following are some examples of the penta-hydroxy and hexa-hydroxy derivatives of β -amyrins.



(XIII)
Theasapogenol E

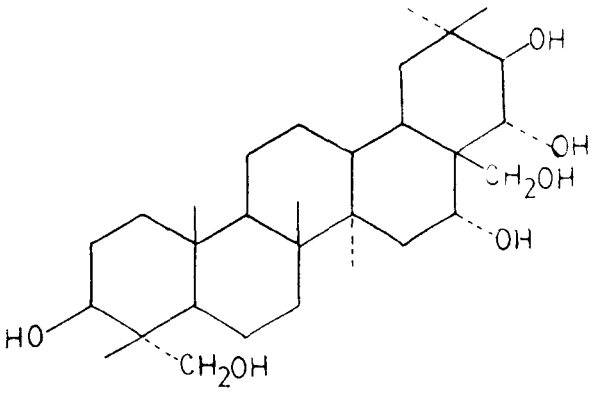


(XIV)
Platicodigenin



(XV)

Aescigenin,proto

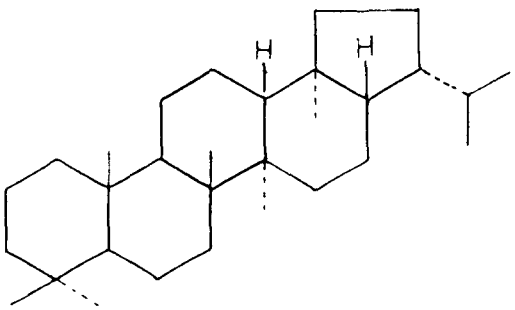


(XVI)

Theasopogenol A

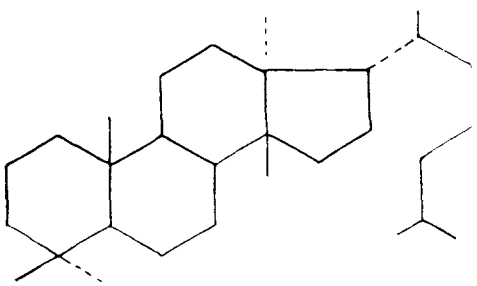
But it is noted that the dihydroxy and trihydroxy derivatives of β-amyrins occur more frequently than the tetra, penta and hexahydroxy derivatives[67]. Such extensive hydroxylation is not seen in &-amyrins or lupeols or in any other triterpenic system. One may therefore conclude that the olean-12-ene system is quite reactive or less sterically hindered so that numerous hydroxy derivatives are formed.

The following chart[68-70] gives some main skeletons of triterpenes occurring commonly.



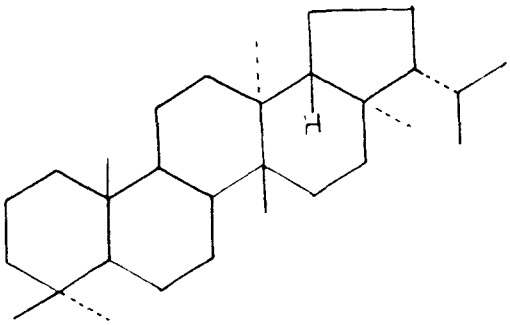
(XVII)

Hopane



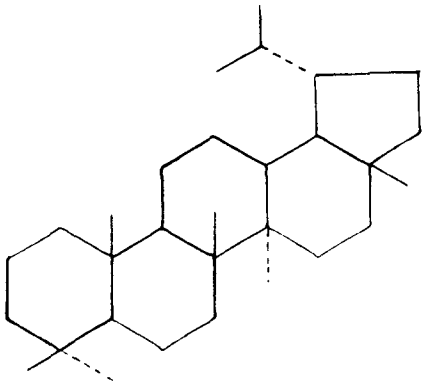
(XVIII)

Euphane



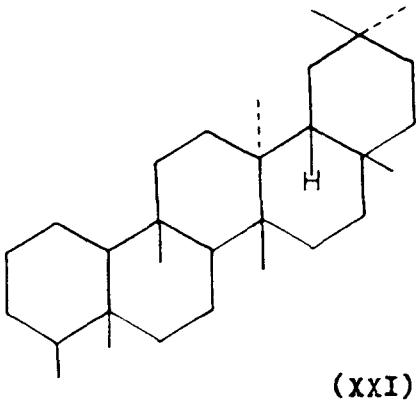
(XIX)

Ferenene



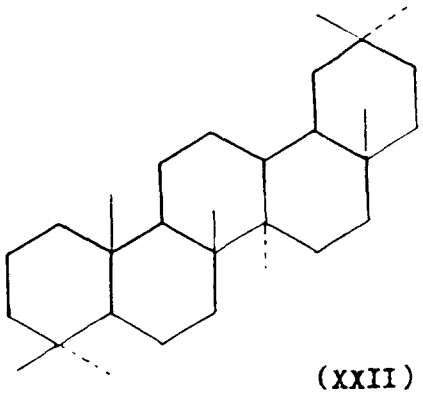
(XX)

Lupane



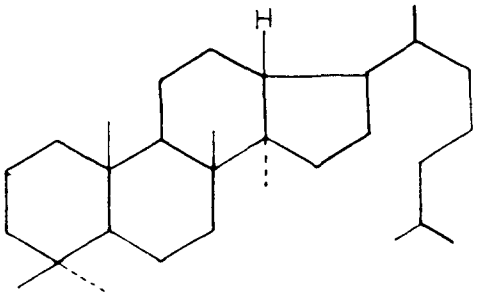
(XXI)

Fridelane



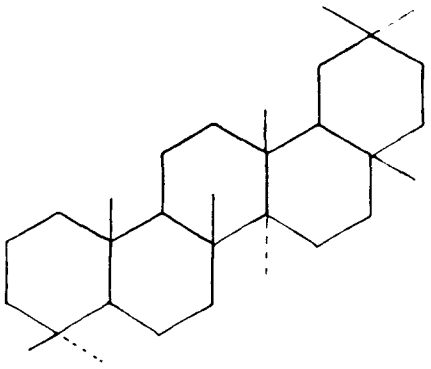
(XXII)

Germanicane



(XXIII)

Dammarane



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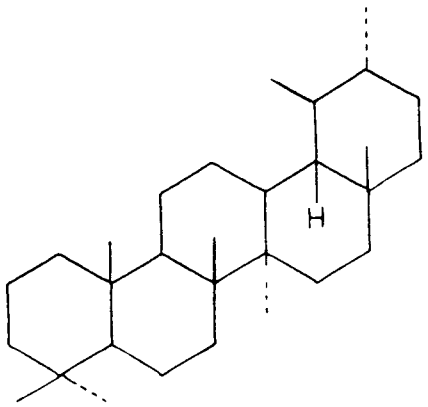
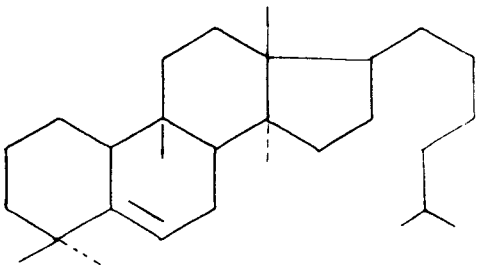
Oleanane



(XXV)
Ursane



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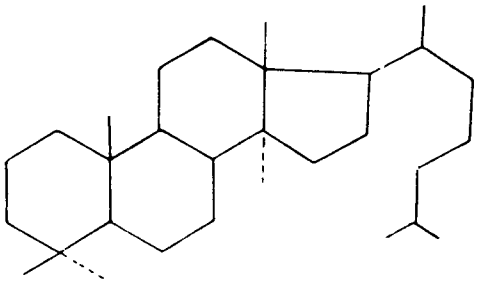

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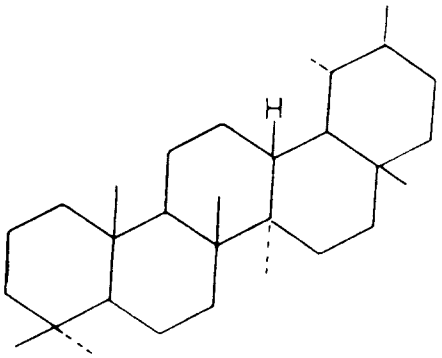

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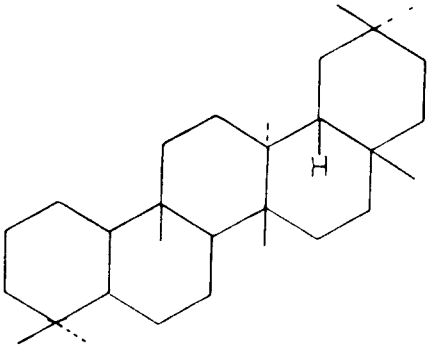

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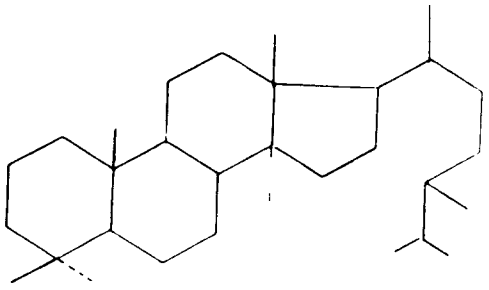

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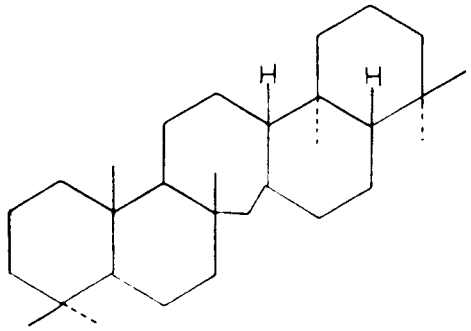

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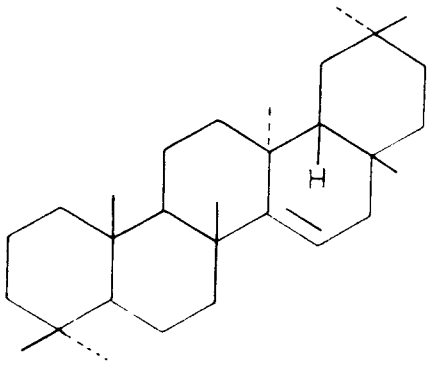

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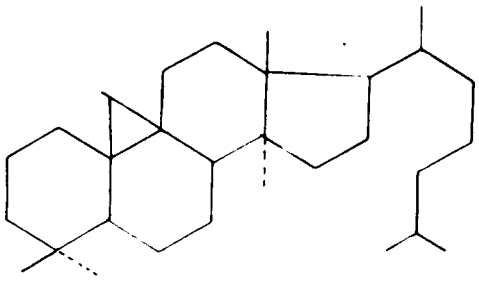

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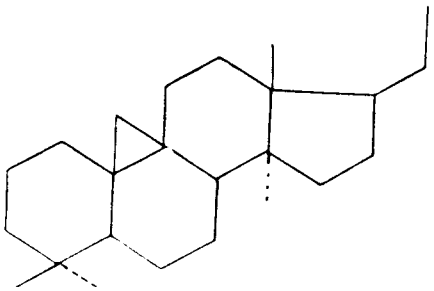

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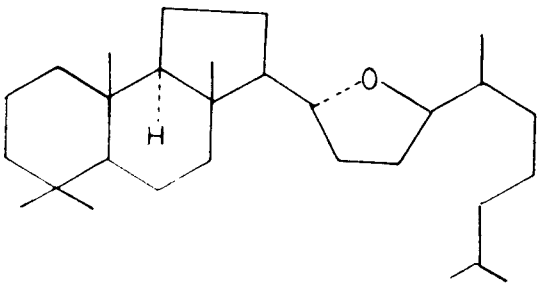

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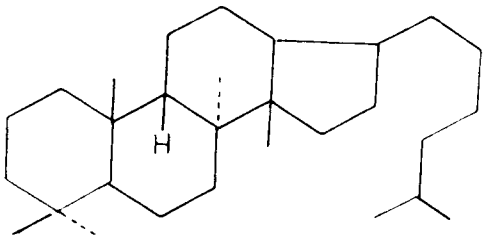

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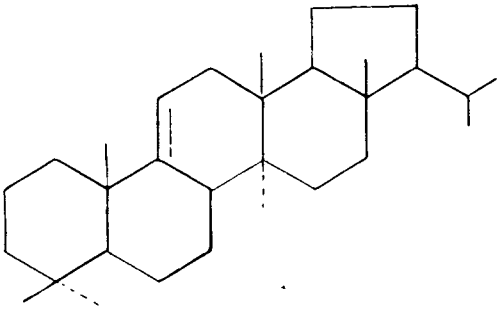

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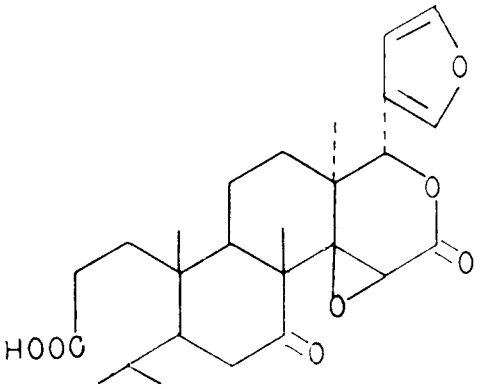

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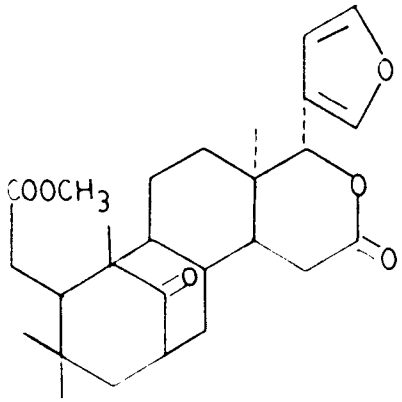

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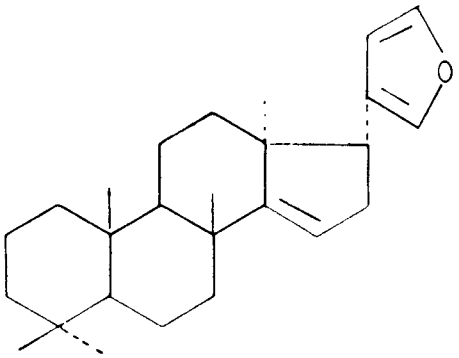

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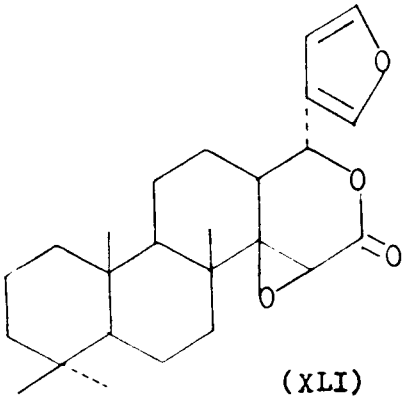

(XLI)

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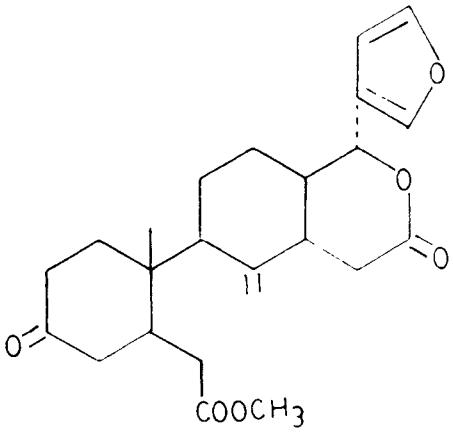
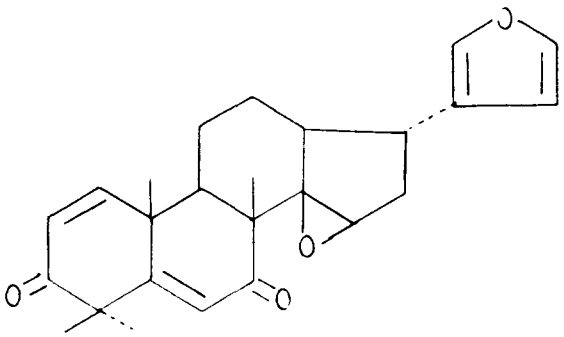


(XLII)

Andirobin



(XLIII)
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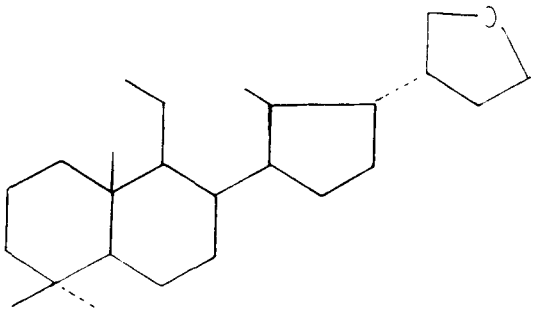

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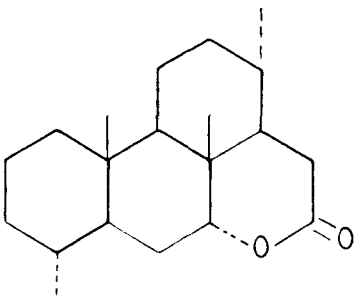

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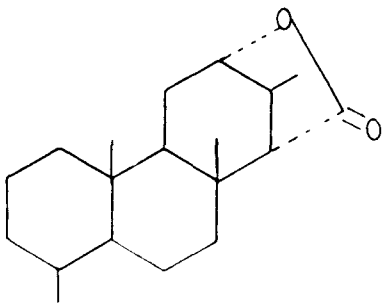

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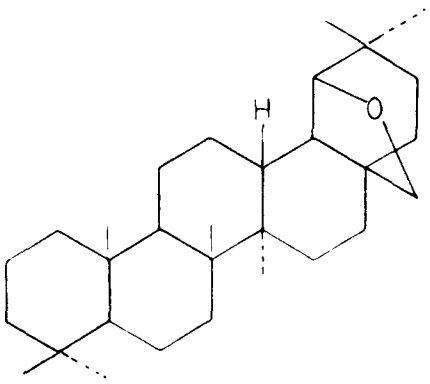

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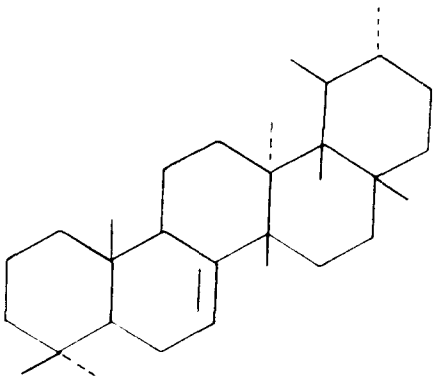

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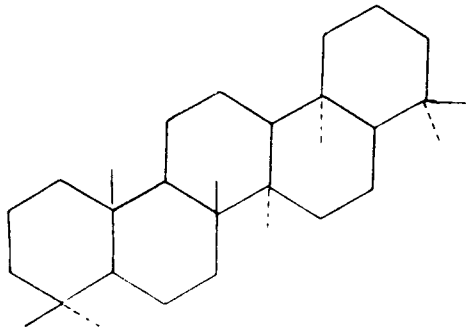

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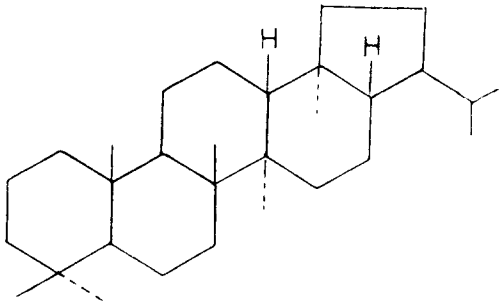
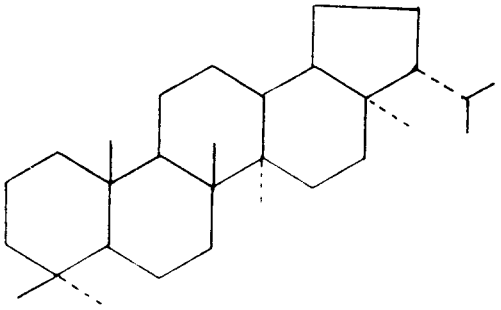


(L)
Moretenol



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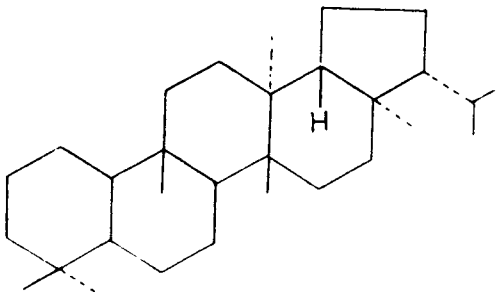

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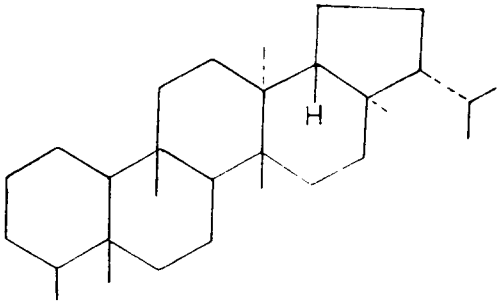
Adianene



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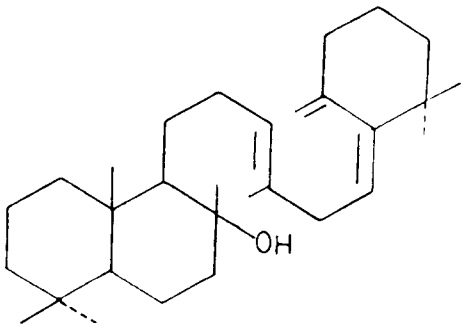
Filisene



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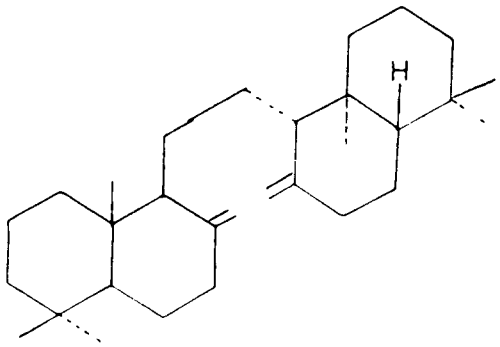
Amberin



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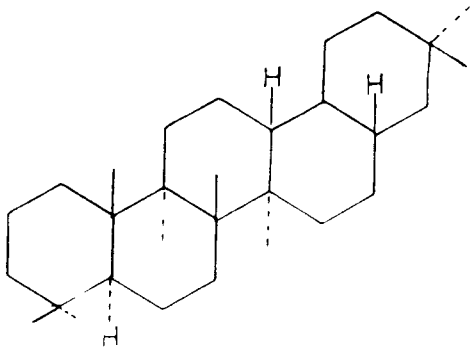
Onocerin



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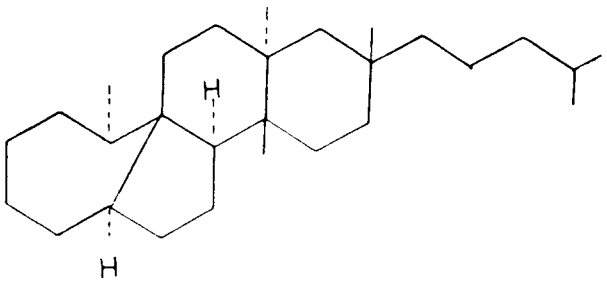
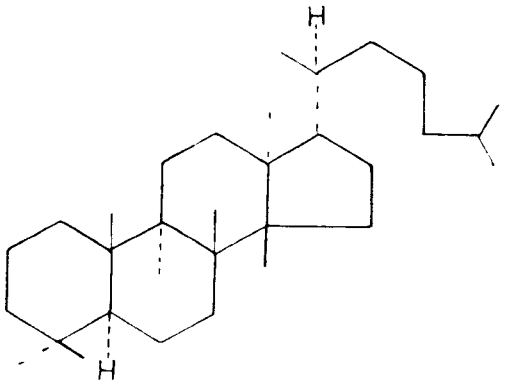


(LVII)
Halimane



(LVIII)
Tirucallane

ISOLATION AND IDENTIFICATION OF TRITERPENOIDS

For the isolation of triterpenes, in general rapidly dried under shade and coarsely powdered plant material is defatted with petrol and then extracted with hot alcohol. Hot alcoholic concentrate is treated with water to give water soluble and water insoluble fractions. Water soluble fraction is subjected to acid or enzymic hydrolysis in order to liberate aglycones, if any glycosides of triterpenoids are present.

Before chromatographic separation the plant extract is purified by various methods like sequential solvent extraction with solvents of increasing polarity (petrol, benzene, chloroform, carbon tetra-chloride, solvent ether, acetone etc.), treatment with activated animal charcoal, fractionation into acid and neutral tri-terpenes by sodium salt formation method, repeated acetylation and deacetylation etc.

A large number of triterpenes were isolated and identified by column chromatography technique. Silica gel column chromatography is frequently used for the isolation of the triterpenoids from petrol and benzene fractions, alumina (neutral or basic)column chromatography is also used many times.

Relatively very little work has been done on the paper chromatographic separation of the triterpenoid mixtures commonly found in plants.

The technique of thin layer chromatography has extensively been used for the separation and characterisation of both acidic and neutral triterpenes. Even simple solvent mixtures like benzene and methylene chloride or hexane and ethyl acetate mixtures give very good separation of neutral triterpenoids on silica gel G-layers. As expected, more strongly polar solvents like diisopropyl-ether, acetone or chloroform-methanol is used for the separation of the triterpenic acids. With some acids, trailing develops, which can be prevented by the addition of pyridine or diethyl amine.

For conducting GLC of triterpenes, liquid phase such as SE-30, OV-1 (methyl siloxane polymer), OF-1 and DEGS (diethylene glycol succinate) may be employed.

A- Colour reactions of triterpenes

1. Salkowski test : A few mg. of the powdered substance dissolved in chloroform on the addition of a few drops of sulphuric acid develops a yellow colour, changing to red.

2. Liebermann-Burchard Test : A few mg. of the substance dissolved in acetic anhydride develops, on the addition of H_2SO_4, a green colour either immediately or through red and blue shades.

3. Rosenthaler Test : The addition of sulphuric acid, to an alcoholic solution of triterpene containing vanillin hydrochloride, develops a lilac colour.

4. Noller Test: The substance (20mg) and 0.5ml. of the reagent (0.1% pure stannic chloride in pure thionyl chloride) is taken in a test tube which is corked and left aside. The solution passes through various colours, but red is always there. The reaction is specific for triterpenes. Oxyacids containing at-least one-OH group give a dark positive colouration.

5. Tetranitromethane Test : The substance (a few mg.) is dissolved in chloroform and a few drops of tetranitromethane solution in chloroform are added. Appearance of a yellow colour indicates the presence of a double bond.

6. Chromatographic Test:[102] A mixture of $SnCl_4$: AcOH:CCl_4 (6:50:50), when sprayed on a filter paper with spots of the substance and heated at 100° produces a brown colour.

7. Whitby Test:[103] To a solution of the substance in chloroform on adding formalin containing a trace of H_2SO_4, varying colours are formed. This test is not given by steroidal sapogenins.

8. Sannie Test[104]: A deposition of a few mg. of the genin on filterpaper when sprayed with an alcoholic solution of cinnamic aldehyde, dried and resprayed with a mixture of acetic anhydride and sulphuric acid develops a yellow colour on heating, indicates the presence of steroidal genins. The triterpenic genins do not respond to this colour reaction.

9. Antimony trichloride reaction[105]: A piece of filter paper dipped in a solution of genin and a solution of $SbCl_3$ in chloroform when treated with a mixture of H_2SO_4 and acetic anhydride, develops orange colour.

A colour reaction[106] has been reported to differentiate triterpenes from steroids based on heating with CCl_3COOH which produces temperature dependant characteristic colours.

B- ANALYTICAL TECHNIQUES

In the field of spectroscopy, the use of ultraviolet and infra red spectroscopy[107,108] is well established since a long time. During the last twenty years application of nuclear magnetic resonance spectroscopy has also rapidly increased. Even more recent has been the application of mass spectroscopy.

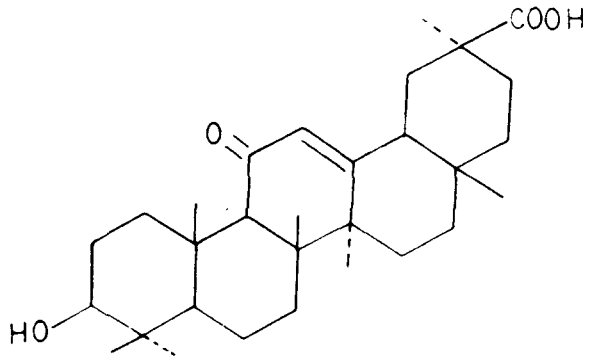
ULTRA-VIOLET SPECTROSCOPY

The U.V. spectroscopy finds its main use in the terpene chemistry for the detection of conjugation. Unsaturated esters, lactones and acids can usually be recognised by their absorption maxima in a particular region. For the prediction of high intensity bands of the system such as conjugated dienes and trienes, conjugated ketones etc., the emperical rules have already been proposed by Woodward[109,110] which can be applied to triterpenes also.

The UV spectra of pentacyclic triterpenes in sulphuric acid shows a characteristic absorption maximum at 310 nm regardless of the substituents present[111]. The hypsochromic shift in the UV spectrum associated with the 18 β to 18 α transformation, observed in the case of α-boswellic acid, has been proposed as a diagnostic technique in conformational analysis[112].

In the case of glycyrrhetic acid (LIX)[113], a consideration of the absorption maximum in the ultra violet region at 250 mμ

Log ϵ 4.1, has suggested that the acid is an α,β-unsaturated ketohydroxy acid.



(LIX)

The triple ultraviolet maxima at 243, 251 and 260 m μ have been found to be characteristic of the typical $\Delta^{11,13(18)}$ dienes of the β-amyrin series, obtained by selenium dioxide oxidation of the compounds. Thus on this basis the members of the β-amyrin series have been distinguished from those of the members of α-amyrin and lupeol groups.

The ultraviolet maxima of α,β-unsaturated lactones and conjugated dienes for a number of compounds in the triterpene series have been discussed and reviewed by Noller[114].

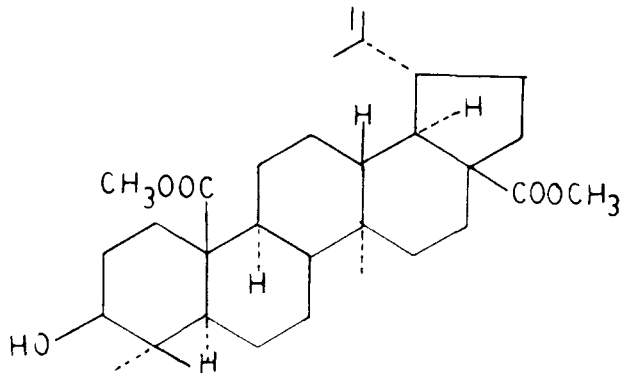
INFRA-RED SPECTROSCOPY

The appearance of the absorption bands in a particular region of the IR spectra and the displacement of these bands due to environmental difference formed the basic principle of infra-red spectroscopic studies. The applicability of such a method,

therefore, depends largely on the availability of reliable data in the light of which the observations in the study of new compounds could be interpreted. Various aspects of the infra-red spectra of steroids have been summarised by many workers[115-117].

The infra-red spectra of triterpenes have got much resemblance with the spectra of steroids, but since the environments at each sub tituent position in the two types of systems i.e. triterpenoids and steroids are not identical, a separate study has, therefore, been made for the triterpenes. For example, for similar positions in C-3 ketones in the series of steroids the C-2 and C-4 methylene groups absorb near $1420cm^{-1}$ while in the corresponding (3-oxo) triterpenes the C-2 methylene group absorbs near $1430\ cm^{-1}$, a C-11 methylene in 12-oxo-steroids absorbs at $1434\ Cm^{-1}$ whereas the same group in 12-oxo-triterpenes absorbs close to $1420\ cm^{-1}$. Cole and co-workers have summarised the positions of carbonyl bands[118], ethylenic double bonds[119] and the equatorial or axial nature of the hydroxyl groups in triterpenic compounds[120] in the IR region.

As a result of infra-red spectroscopic studies, it might be possible to make a distinction between tertiary equatorial ($3613\ Cm^{-1}$) and axial ($3617\ Cm^{-1}$) hydroxyl groups. On this basis the band at $3629\ Cm^{-1}(CCl_4)$ in methyl melaleucate[121](LX) has been assigned as equatorial secondary, while its 3-epimer obtained by oxidation of the ketone and subsequent reduction absorbing at $3636\ Cm^{-1}$ as axial secondary.



(LX)

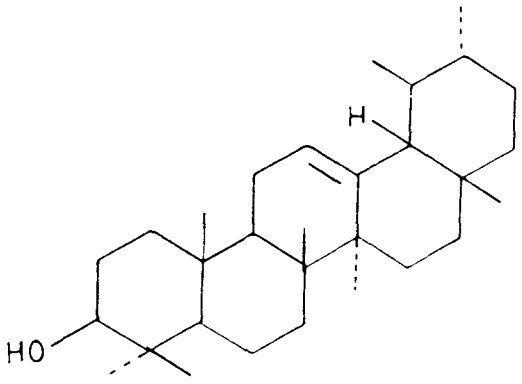
A study of the IR spectra of 18 £-H and 18 β -H olean-12-ene derivatives[122] showed that 18β-H compounds with an axial COOMe absorbed at 1117 Cm^{-1}. The 18 β -H compound with an axial CH_2OH at C-20 absorbed at 1210-1205 Cm^{-1}, whereas the 18 £ -H epimers absorbed at 1193-1189 Cm^{-1}.

NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

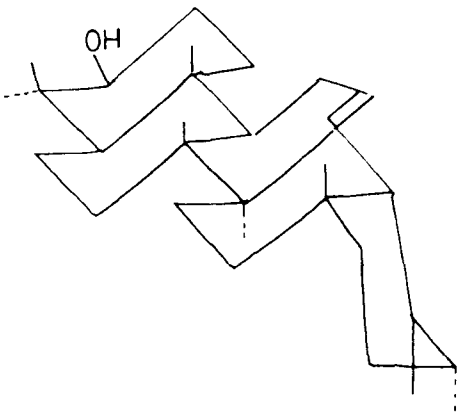
The structure elucidation of a natural product usually involves four steps. The first stage is the determination of emperical and molecular formulae. This is usually followed by the demonstration of the presence of various classes of functional groups. The next stage is to determine the number of functional groups in each particular class. Finally, it is necessary to determine the sequence in which the functional groups and intervening atoms occur within the molecule. Proton magnetic resonance spectroscopy provides useful information right from the second stage to the final establishment of the structure of a compound. Proton magnetic resonance spectra frequently indicates the various classes of protons present in the molecule.

Once the structure has been defined, the question of relative stereochemistry of the molecule has to be established, and here again NMR spectroscopy can be most useful.

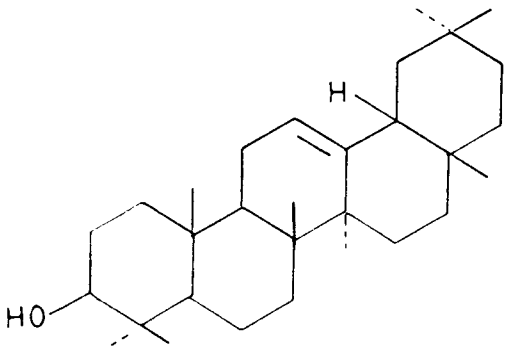
NMR spectroscopy has provided a great help in the structure elucidation of triterpenes. Since the oleanane, ursane and lupane series are the most wide spread of pentacyclic triterpene found in nature, the majority of triterpenes studied here belong to these three groups. So the present study of the NMR spectra of pentacyclic triterpenes was initiated by Maurice Shamma, Richard E. Glick and Ralph O. Mumma, will help in structure elucidation of new pentacyclic triterpenes. The simplest alcohols of the ursane, oleanane and lupane series α-amyrin (LXI), β-amyrin (LXII) and lupeol (LXIII) respectively are studied in detail. In an effort to increase the solubility of the triterpenes and make the spectra more significant, most of the triterpenes are converted to their corresponding methyl ester acetate derivatives.



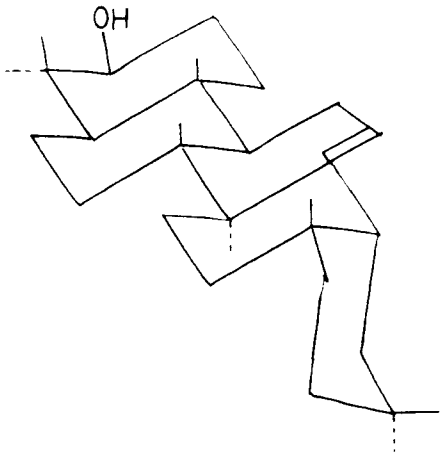
(LXI)



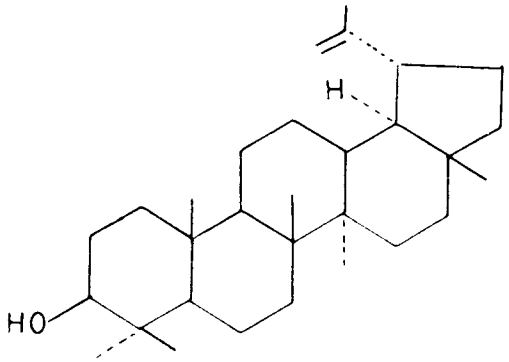
(LXI a)



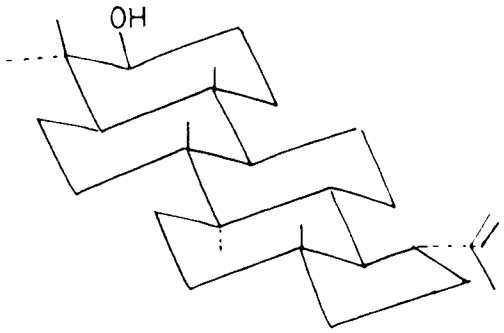
(LXII)



(LXIIa)



(LXIII)



(LXIIIa)

SOME CHARACTERISTICS OF THE NMR SPECTRA OF PENTACYCLIC TRITERPENES

Many distinct absorptions can be found in the spectra of pentacyclic triterpenes. Methyl esters and acetoxyl groups give sharp absorptions[123]. Angular methyl groups also give well defined absorptions. However, since pentacyclic triterpenes contain a number of such methyl groups, their absorptions often are found to overlap.

Certain other functional groups such as vinylic protons, protons alpha to hydroxyls or acetoxyls and methylene protons, all were found to have low and diffuse absorptions. Even so, these absorptions are important because they give a clue to certain structural features of the triterpenes.

Chemical Shift of the Highest C-Methyl Group

Whenever a carbomethoxyl function is present in the molecule, it was noticed that the chemical shift of the highest (most shielded) C-methyl group is partially indicative of the position of the carbomethoxyl. Thus, in every case in which a C-28 carbomethoxyl function is present in a triterpene of the ursane or oleanane series, the highest C-methyl absorption peak appears upfield from 0.775. Alternatively, when the C-28 position is represented either by hydroxymethylene, a methyl group, or a lactone, the highest C-methyl absorption peak appears downfield from 0.775.

A few of the triterpenoids were found to have their highest C-methyl absorption close to the dividing line ; however, most were far to one side or the other. Friedelin is the only naturally occurring triterpene which has its highest C-methyl absorption

below 0.775 and which did not possess a C-28 carbomethoxyl function.

Following table gives the chemical shifts of highest C-methyl groups :

TABLE -2

CHEMICAL SHIFTS OF HIGHEST C-METHYL GROUPS

Triterpenes	Chemical shifts	C-28 carbomethoxyl functions
Arjunolic acid methyl ester triacetate	0.683	Yes
Oleanolic acid methyl ester acetate	0.715	Yes
Oleanolic acid methyl ester	0.730	Yes
Ursolic acid methylester acetate	0.735	Yes
Glycyrrhetic acid methyl ester acetate	0.838	No
α - Amyrin benzoate	0.865	No
β - Amyrin benzoate	0.905	No
Lupanol	0.803	No
Betulin diacetate	0.840	No
Friedelin	0.703	No

Methyl Ester Absorption

The position of the absorption of the methoxyl moiety of a methyl ester is also partially indicative of the relative position of the carbomethoxyl group in the tri-terpene molecule.

Ester rule : The absorption of C-28 methyl ester belonging to the oleanane or ursane group is usually upfield from 3.595 while the carbomethoxyl located in other positions such as at C-24 or at C-30 absorb further downfield in the region from 3.595 to 3.650.

Therefore, there are two ways to verify the presence of a C-28 carbomethoxyl group in an ursane or oleanane triterpene, namely, from the chemical shift of the highest angular methyl and from the position of the methoxyl group absorption. Ester of oleanane and ursane were found to obey the ester rule mentioned above (Table 3).

TABLE- 3

ABSORPTION OF METHYL ESTERS

Triterpenes	Methoxyl Absorptions	Position of carbomethoxyl function
Ursolic acid methyl ester	3.578	C-28
Ursolic acid methyl ester acetate	3.535	C-28
Asiatic acid methyl ester triacetate	3.538	C-28
Arjunolic acid methyl ester triacetate	5.538	C-28
Oleanolic acid methyl ester acetate	3.555	C-28
Cochalic acid methyl ester	3.570	C-28
Oleanolic acid methyl ester	3.580	C-28
Glycyrrhetic acid methyl ester	3.645	C-30
11-Keto-β-boswellic acid methyl ester acetate	3.597	C-24

As in the case of the C-methyl absorption, the reason for the differences in the positions of the methyl ester absorptions is not known. It may be related to the fact that the C-28 carbomethoxyl function is extremely hindred or that this functional group is influenced by the magnetic anisotropy of the 12(13) double bond.

Vinyl Proton Absorption

The proton of the normal trisubstituted double bond in the ursane and oleanane series absorbs in the region between 4.93 and 5.50. This absorption is broad and its center is poorly defined. However, if the double bond is conjugated with a carbonyl function at C-11, such as in 11-keto-β boswellic acid methyl ester acetate the vinylic proton is found to absorb lower field i.e. at 5.55 and the peak becomes much sharper. This downfield shift is to be expected since the keto group is electron withdrawing and causes the vinyl proton to be less shielded while the sharpening of the peak can be easily explained by the absence of any alpha hydrogens.

If a terminal double bond is present, such as in the lupane series, the vinyl protons absorb at higher field, i.e. around 4.30 to 5.87. This type of vinylic absorption is easily recognized by its larger size since it represents two protons.

Vinylic Methyl Absorption

Another useful and characteristic absorption was found to be that of the vinylic methyl function, CH_3-C=C. Normal methyl groups absorb from 0.625 to 1.500. Vinylic methyl peaks usually appear

between 1.63 and 1.80 and are sharp and well defined.

Acetoxyl Absorption

Acetoxyl protons give the sharpest absorption of any function in the triterpene series. This absorption usually appears between 1.82 and 2.07, with majority of such protons absorbing between 1.92 and 1.97. Since acetoxyl peaks are sharp and clear a difference of even 0.02 p.p.m. between two peaks can still be recognized.

Protons Alpha to Secondary Acetoxyl Groups

The absorption of protons alpha to acetoxyl groups usually appears as a broad hump. This type of absorption may be grouped into two classes depending on whether the acetoxyl group is primary or secondary.

A proton alpha to a secondary acetoxyl group gives an absorption which is about 30 c.p.s. broad. However, this peak is so low in intensity that unless a concentrated solution is used the signal may be obscured by the noise.

It was found that the axial C-3 proton of an acetylated triterpene absorbs between 4.00 to 4.75. The corresponding equatorial proton, as in α-boswellic acid methyl ester acetate or its 11-keto derivative, absorbs some twentynine cycles beyond the axial at 5.00 to 5.48 and does not exhibit as broad an absorption as the axial proton. Shoolery and Rogers[124] noted this same effect in the steroids, namely, that the equatorial protons showed up at lower field than the axial. If a strong 1,3-interaction is present between axial proton and an angular methyl group, that axial proton will absorb at lower field[125].

Protons Alpha to Acetylated 1,2 Glycols

Triterpenes often contain 1,2-glycol functions and most frequently these are found at C-2, C-3, and at C-15, C-16. It was found that the protons alpha to an acetylated 1,2-glycol appear at much lower field than the protons alpha to isolated acetoxyl groups, and give sharper peaks, with areas indicating two protons. In the present study four triterpenes were studied containing acetylated trans diequatorial vicinal glycol systems, and the absorptions are recorded in the Table 4.

TABLE -4

ABSORPTIONS OF PROTONS ALPHA TO ACETOXYL GROUPS IN ACETYLATED 1,2-GLYCOLS

Triterpenes	Widths of absorptions	Peaks	Positions of acetoxyl groups
Arjunolic acid methyl ester triacetate	4.70-5.18 (19 c.p.s.)	4.98	2,3
Asiatic acid methyl ester triacetate	4.70-5.18 (19 c.p.s.)	5.00	2,3
A_1-Barrigenol pentaacetate	5.23-5.69 (16 c.p.s.)	5.54	15,16
7 β-Hydroxy-A_1-barrigenol hexaacetate	5.25-5.64 (19 c.p.s.)	5.36	15,16

Protons Alpha to Primary Acetoxyl Functions

The methylene protons of an acetoxymethyl group may absorb as a sharply defined singlet, doublet or quartet probably depending upon the degree of steric hindrance. The position of the absorption was found to vary over a wide range depending on the relative position of the methylene group in question. Table 5 lists the

triterpenoids containing acetoxymethyl functions and the corresponding absorptions. Hence nuclear magnetic resonance spectroscopy can be of definite use in establishing the position of acetoxymethyl groups.

TABLE - 5

ABSORPTION OF METHYLENE PROTONS OF ACETOXYMETHYL GROUPS

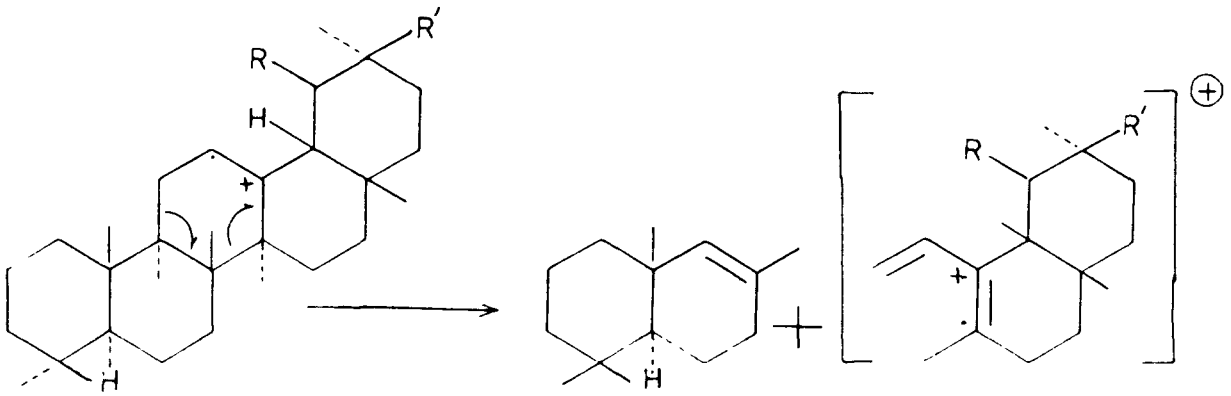
Triterpenes	Absorptions	Types	Positions
Arjunolic acid methyl ester triacetate	3.65	s.	23
Asiatic acid methyl ester triacetate	3.65	s.	23
A_1-Barrigenol pentaacetate	3.82	q.	28
11-Keto-A_1-barrigenol pentaacetate	3.85	q.	28
Erythrodiol diacetate	3.85	q.	28
Betulin diacetate	4.05	q.	28
A_1-Barrigenol pentaacetate	5.08	d.	27
7 β -Hydroxy-A_1-barrigenol hexaacetate	5.18	s.	27

s. = singlet, d.=doublet and q.= quartet

MASS SPECTROSCOPY

Mass spectroscopy is an excellent method for finding out the molecular weights of organic compound. Along with other spectral information, the mass spectroscopy is a tool for the determination of the structure of compounds. It has unique advantages over other techniques.

The reports on the mass spectral studies of large number of tetracyclic and pentacyclic triterpenoids are available[25]. A detailed study of the systematic cracking pattern of pentacyclic triterpenes by noting peak shifts in various derivatives was very well carried out by C. Djerassi et al.[126-127] and J.S. - Shannon[128] in 1963. This led to important generalisations about triterpenic compounds particularly of Δ^{12}-oleanene and Δ^{12}-ursene derivatives[128-139]. With the unsaturated triterpenes, one of the characteristic fragmentation is a retro-diel-alder reaction[140] i.e. abstraction of an electron from the olefinic π bond, followed by a R-D-A type opening of ring C shown in given scheme 1.



(a) (b)

R=CH_3; R' = H or R=H; R' = CH_3

SCHEME 1

The mass of fragments provides considerable information about the position of double bonds and substituents. Triterpenes have been examined by several workers C.Djarassi et.al[126-127], J.S.Shannon R.O.Donchaf et.al[131] and several other workers[141-143], who have shown that there are important characteristics of the group as well as of the individual members. Some of the most important fragmentation include dehydration of its equivalent (M^+-SC). In Δ^{12} oleanene and Δ^{12}-ursene derivatives, oxygen containing substituent at C-17 or C-3 ($COOH_3$' CH_2OH,CH_2OAc or CHO) are lost in preference to methyl from R-D-A fragmentation owing to stabilisation of the expelled radical by oxygen, 12 oxygenated triterpene exhibit base peak at m/e 234.

The process of fragmentation of basic triterpene of β-amyrin and α-amyrin series hydrocarbons was shown by taking example of Δ^{12} -oleanene and Δ^{12}-ursene described by Jerry Karliner and Carl - Djerassi[144] in 1966 is as follows :

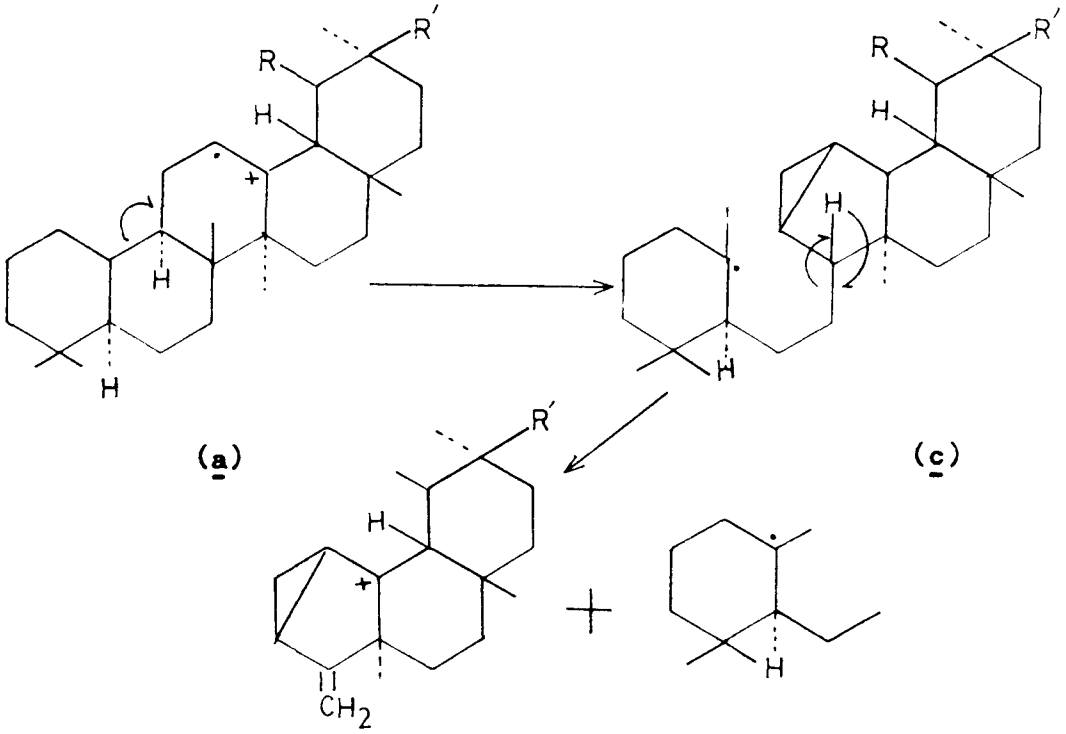
In the mass spectra of these compounds it can be seen that they give rise to fragments at identical m/e values. But fragment ion m/e 203 is more intense than m/e 191 in case of Δ^{12} oleanene and reverse is seen in Δ^{12} ursene. This peak can be utilized to differentiate between two.

The Important Peaks of Triterpenes:

Peak M-153 (m/e 257) results from the homolytic cleavage of 9-10 bond in the molecular ion (a) to afford (c) followed by hydrogen transfer from C-26 to C-7 with concomitant homolysis of 7-8 bond to

afford the resonance stablised species (d) (m/e 257), below 2:

SCHEME NO.2

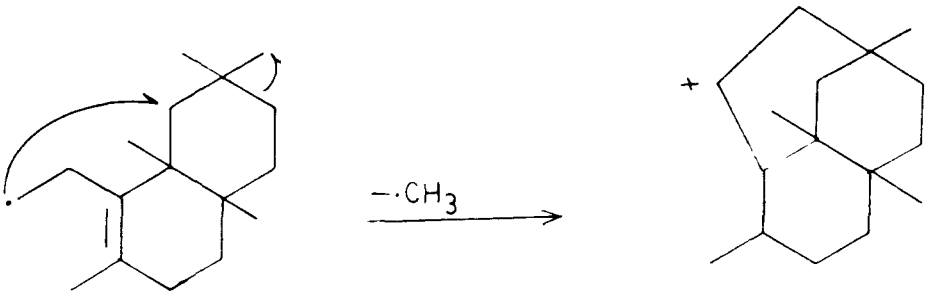


(d) m/e 257 $R=CH_3$; R'=H or R=H; $R'=CH_3$

PEAK M-192 (m/e 218) The intense and diagnostically important m/e 218 peak, base peak (in Δ^{12} oleanene and Δ^{12}-ursene) has been discussed in considerable detail earlier[126,127,140] Mechanism of formation of m/e 218 species by R-D-A decomposition is already discussed and shown in Scheme 1.

PEAK M-207 (m/e 203) This is the most interesting peak from mechanistic point of view and may result from fragmentation described in schemes given below :

Mechanistic interpretation of M/e 203 in Δ^{12}-oleanene Series - Scheme 3, m/e 203, may result from further loss of 15 mass units from the retro-diels-alder fragment (b) to yield -



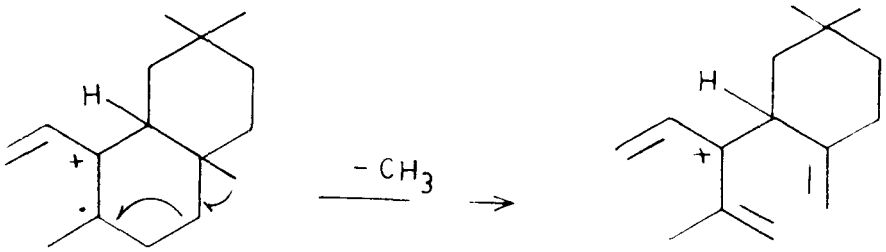
(b) (e), m/e 203

SCHEME-3

From various studies it has been concluded that this path way does not represent a significant pathway for genesis of the m/e 203 species.

m/e 203 moiety can also be obtained from R-D-A fragment (b) by equal loss of methyl substituents at C-17 or C-20 by given schemes:

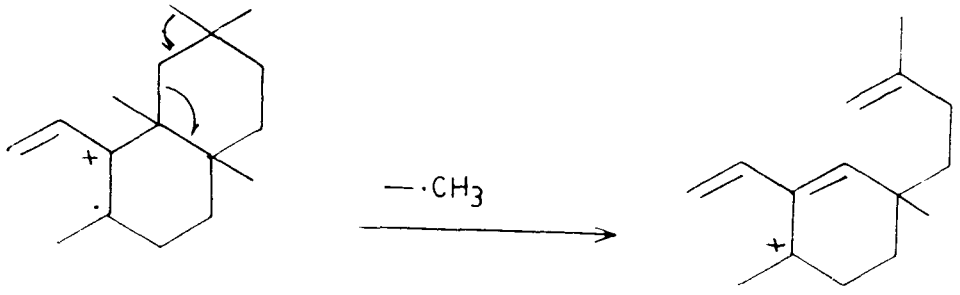
Scheme 4 fragment (b) may suffer a loss of the C-28 methyl group while undergoing homolytic scission of 15-16 bond to afford, the resonance stabilised ↔ destabilised dienyl cation (f)



(d) (f) m/e 203

SCHEME-4

Scheme 5 : Expulsion of methyl group bonded to C-20 (either C-29 or C-30methyl group) with concomitant homolytic cleavage of the 18-19 bond results in dienyl cation (g).

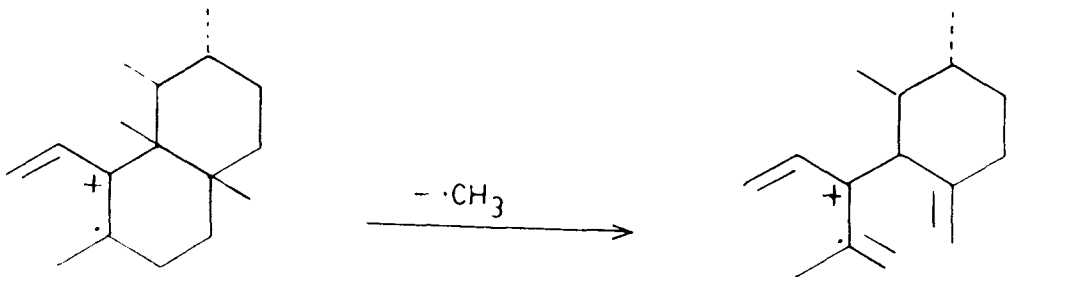


(b) (q) m/e 203

SCHEME - 5

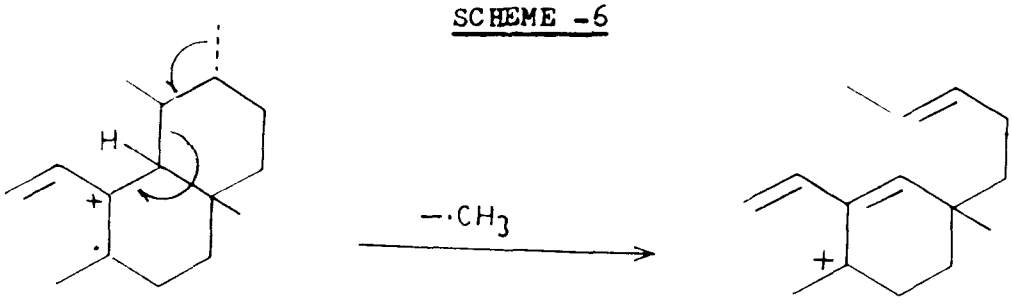
Mechanistic Interpretation of m/e 203 in Ursene Series

Formation of m/e 203 moiety may result from 50 loss of C-28 methyl radical and 50 expulsion of C-30 radical (since ursene contain only single group. at C-20, C-28 expulsion accounts for only 35 for the formation of m/e 203 moiety). So in ursene all the methyl groups of ring E contribute to same extent and lead to following three pathways:



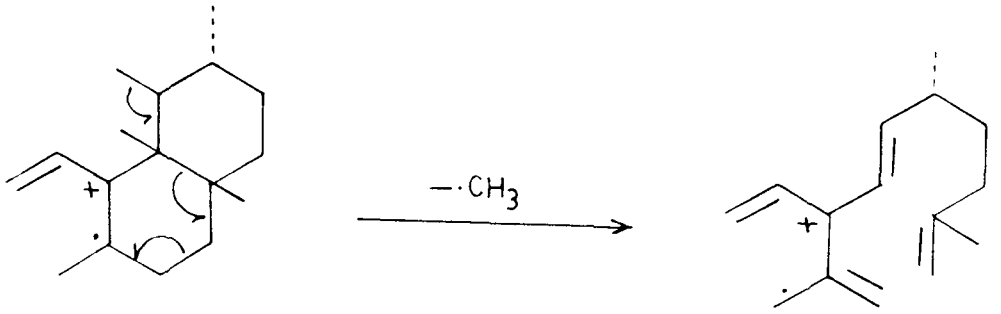
(b) (h) m/e 203

SCHEME -6



(b) (i) m/e 203

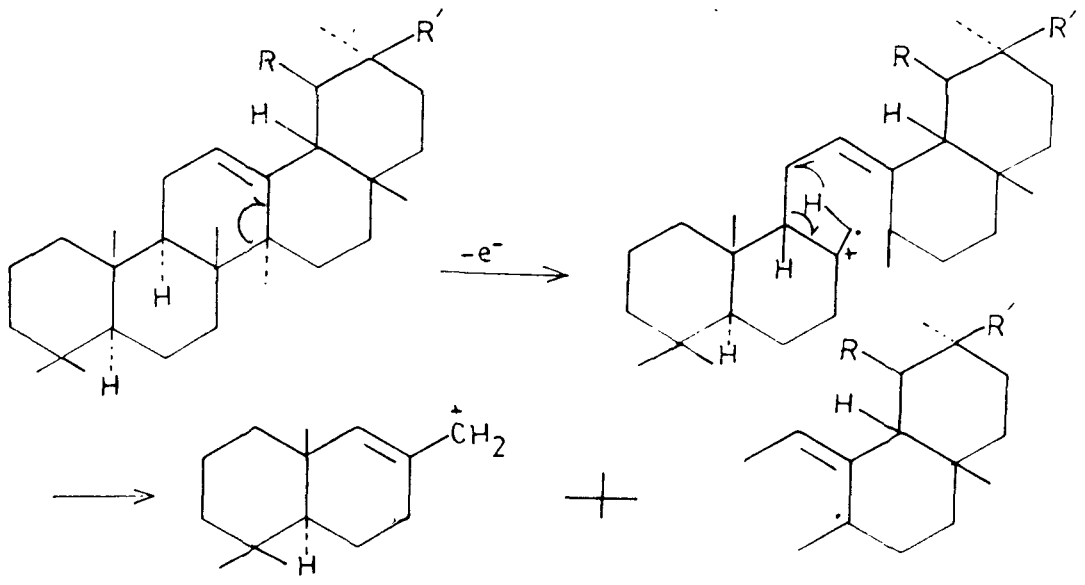
Above two pathways are analogus to Δ^{12} oleanene as in (f) and (q) while third one, (b)-(j) involves expulsion of C-29 methyl radical and homolytic fission of the 17-18 and 15-16 bond.



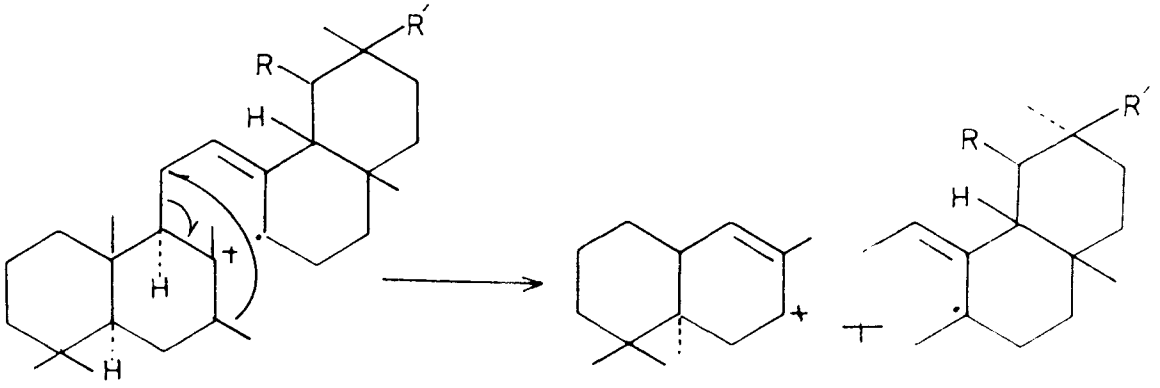
(b) (j) m/e 203

SCHEME -8

PEAK M-219 (m/e 191)-mechanism proposed[126-127] for this species involved heterolytic fission of the allylically activated 8-14 bond to from (k), consisting of a tertiary carbonium ion and an allylic radical. Hydrogen transfer from C-26 to C-11 with simultaneous bond breaking at 9-11 would result in formation of (l) m/e 191.



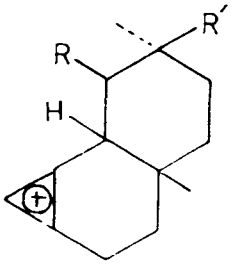
Such peak also result from similar hydrogen transfer from C-7 as from C-26 to C-11 (prefered as transfer of secondary vs. a primary hydrogen atom)[145] and (k) ⟶ (m) is energetically more favourable than (k) ⟶ (l).



(k) (m), m/e 191

R = CH_3; R' = H or R= H; R' = CH_3

Peak M-221 (m/e 189)- This fragment occuring at m/e 189 has been found more complicated than thought earlier[126,127]. A structure (n) a stable cylopropeniun cation as a representation of the m/e 189 fragment has been proposed although its manner of formation is not obvious.



(n) m/e 189

R=CH_3; R' = H or R= H; R' = CH_3

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NEW WORK

CHAPTER-I

DISCUSSION

STUDY OF THE FLOWERS OF ICHNOCARPUS FRUTESCENS R.Br.

SYN: APOCYNUM FRUTESCENS LINN. (N.O. APOCYNACEAE)

VERNACULAR NAMES

Hindi : Kali dudhi, Dudhilata, Siamlata, English: Black creeper, Sanskrit : Syamlata, Sariva, paravalli, Bengali : Syamlata, Dudhi, Malyalam : Palvali, Marathi : Kantebhouri, Krishnasarwa, Tamil: Udarkodi, Illu-Katta, Kannad : Kare-hambu. Telgu: Illukkatte, Nellatiga, Local - Name (Moradabad District)- Boan.

DISTRIBUTION

It is a climbing plant found almost in all parts of India, ascending to a height of 1,200 m[1,2].

BOTANICAL DESCRIPTION

It is an evergreen, extensively climbing and much branched shrub. Young branches, inflorescences and petrioles are rustly-villous. Leaves are variable, elliptic oblong to broadly lanceolate. Flowers are greenish - white, somewhat fragrant and about 5x4 mm in size and are found in winter. Corolla is two times long as the calyx, tube swollen round the included anthers; lobes twisted -acuminate.[1,2]

MEDICINAL PROPERTIES AND USES

Its roots are used as demulcent, alterative, tonic, diuretic, diaphoretic and as a substitute for Indian Sarsaparilla (Hemidesmus indicus). Leaves and stalks are used in the form of a

decoction in fever[1]. The roots are used in skin diseases[3] and if tied around the neck are said to induce sound sleep[4]. The palnt is considered sueful by the tribals (Santals) in night blindness, bleeding gums, ulcerated tongue, sores, enlargement of spleen, atrophy, cachexia, convulsions, delirium, measles, small pox, haematuria, dysentery, cough, phthisis, dog bite, snake bite and spider-lick[5].

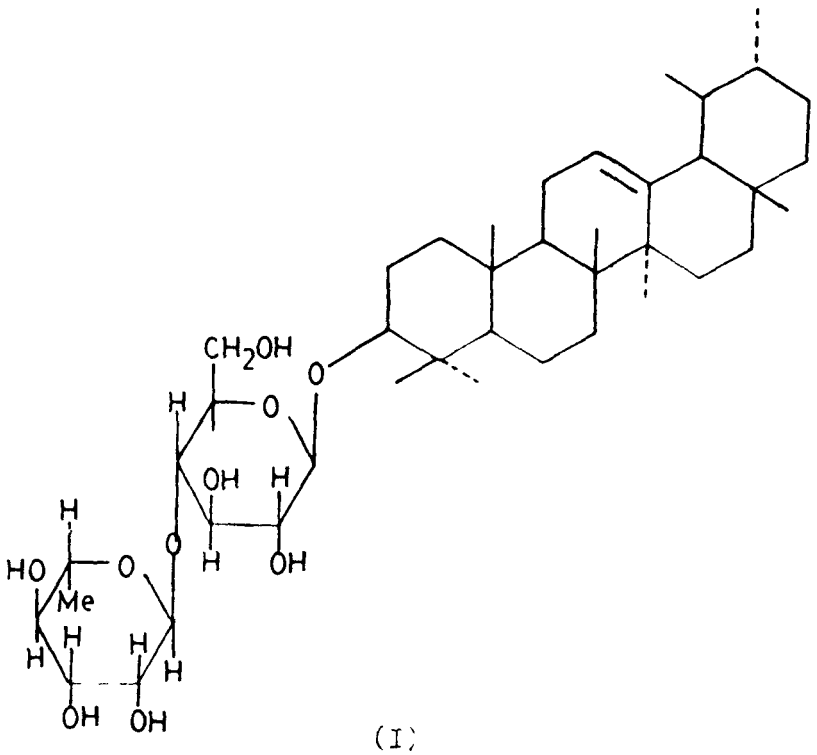
LITERATURE SURVEY OF THE PAST WORK

The alcoholic extract of the plant did not show anti-bacterial, antifungal, antiprotozoal, anticancereus, anthelmintic and hypoglycemic actions and no effect was observed on smooth muscle preparations also. But 50% alcoholic extract of the whole plant was found to have antiviral properties against Ranikhet disease virus and had no effect on vaccinia virus[6]. The extract did not show any effect on respiration and blood pressure in cat/dog. The production of hypothermia and gross behavioural changes have not been reported on administration of the extract in mice. 1gm/kg of the extract was found to be the maxinum tolerated dose in mice.[6]

Quercetin and quercetin -3-B-D-glucopyranoside have been isolated from the ethyl acetate extract of the fresh flowers[9]. Occurence of B-sitosterol[7] has been reported in the roots and apigenin, luteolin, syringic acid, vanilic acid, protocatechuic acid and sinapic acid have been reported in the leaves[8].

α-amyrin, α-amyrin acetate, lupeol, lupeol acetate, friedelin, 3-friedelanol, β-sitosterol have been reported from the petroleum ether extract of the stem[11], another compound, 3,4-trimethyl-octacosan-7-one has also been reported from its stem[12].

From stem of the plant one triterpenic compound has been isolated by Mahocha and Taylor[10]. This new compound has been characterized as α-L-rhamnopyranosyl-(1$\rightarrow$4)-β-D-glucopyranosyl-(1$\rightarrow$3)-α-amyrin (I).



(I)

From the petroleum ether extract of the stem bark of *Vernonia tashiensis* the following compounds were isolated, namely, lupeol, lupeol acetate, friedelin, friedelinol, 3β-hydroxyolean-12-ene, behenic acid, nonacosane 5-hydroxyoctacosan-25-one, dotriacontanoic acid, sitosterol and sitosterol palmitate which were characterized on

of Δ^{12}-dehydrolupanyl 3-β-palmitate and 5-hydroxy-octacosan - 25-one were reported for the first time in nature.

As this plant is quite interesting and considered valuable in indigenous medicine, it was considered worthwhile to re-examine it, and hence these studies were initiated.

PRESENT WORK

The fresh flowers were collected from Sanai (a village in Tehsil Bilari of Distt. Moradabad(U.P.) and dried under shade. The botanical identity of the collected material was established with the help of the Department of Botany, University of Delhi, Delhi. All dried powdered flowers were exhaustively extracted with ethyl alcohol in a soxhlet extractor. The alcohol was recovered and the residue was extracted with petroleum ether several times.

STUDY OF THE PETROL SOLUBLE FRACTION

All the petrol extracts were combined together and after concentrating to a small volume left in a refrigerator overnight. A green solid separated out, which was filtered and washed with petrol. It gave a positive L.B. test and on TLC examination showed two spots (one major and one minor) indicating it to be a mixture. The failure of a number of attempts at crystallisation from different solvents also suggested the nature of the compound to be a mixture and, therefore, it was refluxed with a solution of alcoholic potassium hydroxide and extracted with ether. Evaporation of the ethereal layer gave the neutral compound while the alkaline solution, on treatment with an excess of hydrochloric acid, gave precipitate of an acidic compound. The quantity of the neutral compound was inappreciable and hence it was not studied further.

The acidic compound gave an acetate, which on crystallisation from methanol had m.p. 254-56°. It gave a positive L.B. test. The deacetylation of the acetate gave the parent acid m.p. 288-90°.

A study of the NMR spectrum of the acetate showed the presence of 7 methyl functions from δ 0.7 to δ 1.0 5. There was one acetoxyl function as a singlet at δ 2.0 and a triplet centred at δ4.5 for a proton δ to the acetoxyl and a multiplet at δ 5.3 characteristic of the Δ^{12} proton of ursene or oleanene series. On methylation with diazomethane, the acetate gave an acetyl methyl ester m.p. 242°. The NMR spectrum of the acetyl methyl ester showed the presence of 7 methyl functions between δ 0.7 to δ 1.02. There were two singlets at δ 2.0 and δ 3.55 accounting for one acetoxyl and one ester methoxyl function respectively, a signal centred at δ 4.5 arising from a proton $\mathcal{L}$ to the acetoxyl and another signal centred at δ 5.25 arising from a Δ^{12} olefinic proton.

On the basis of these data it could be concluded that the parent compound is a monohydroxy monocarboxylic acid belonging either to Δ^{12} ursene or Δ^{12} oleanene series of triterpenoids. The relationship with the Δ^{12} ursene group was established by the examination of the U.V. spectrum of the product of oxidation of the acetate with selenium dioxide. The product obtained did not show the characteristic triple ultraviolet absorption maxima (λ_{max}^{EtOH} 241, 249, 255 mμ) of a diene and hence it was concluded that the parent compound may be ursolic acid.

The mass spectrum of the acetate and its methyl ester showed the M^+ peaks at m/e 498 and 512 respectively. The peaks at

m/e 249 and m/e 248 in the acetate are due to the fragments (A) and (B), the fragment (A) arising from rings A/B and the fragment B arising from D/E rings.

Similarly in the mass spectrum of the acetyl methyl ester the fragments (A) and (B) gave rise to peaks at m/e 249 and 262 respectively, which by loss of 60 mass units gave peaks at m/e 189 and 203 respectively. In the following charts (C-1) and (C-2) the fragmentation patterns of the acetyl derivative and acetyl methyl ester have been shown. Final identity of the parent compound as ursolic acid was established (i) by comparing the IR spectrum of the acetyl methyl ester with acetyl methyl ursolate which were superimposable; and (ii) TLC examination of the parent compound alongside with authentic samples of ursolic acid and oleanolic acid in the solvent system: petroleum ether, ethyl formate, formic acid (93:7:0.7) which is specific for distinction between ursolic acid and oleanolic acid[14]. The Rf value (0.19) of the compound compared well with ursolic acid and thus the identity was established.

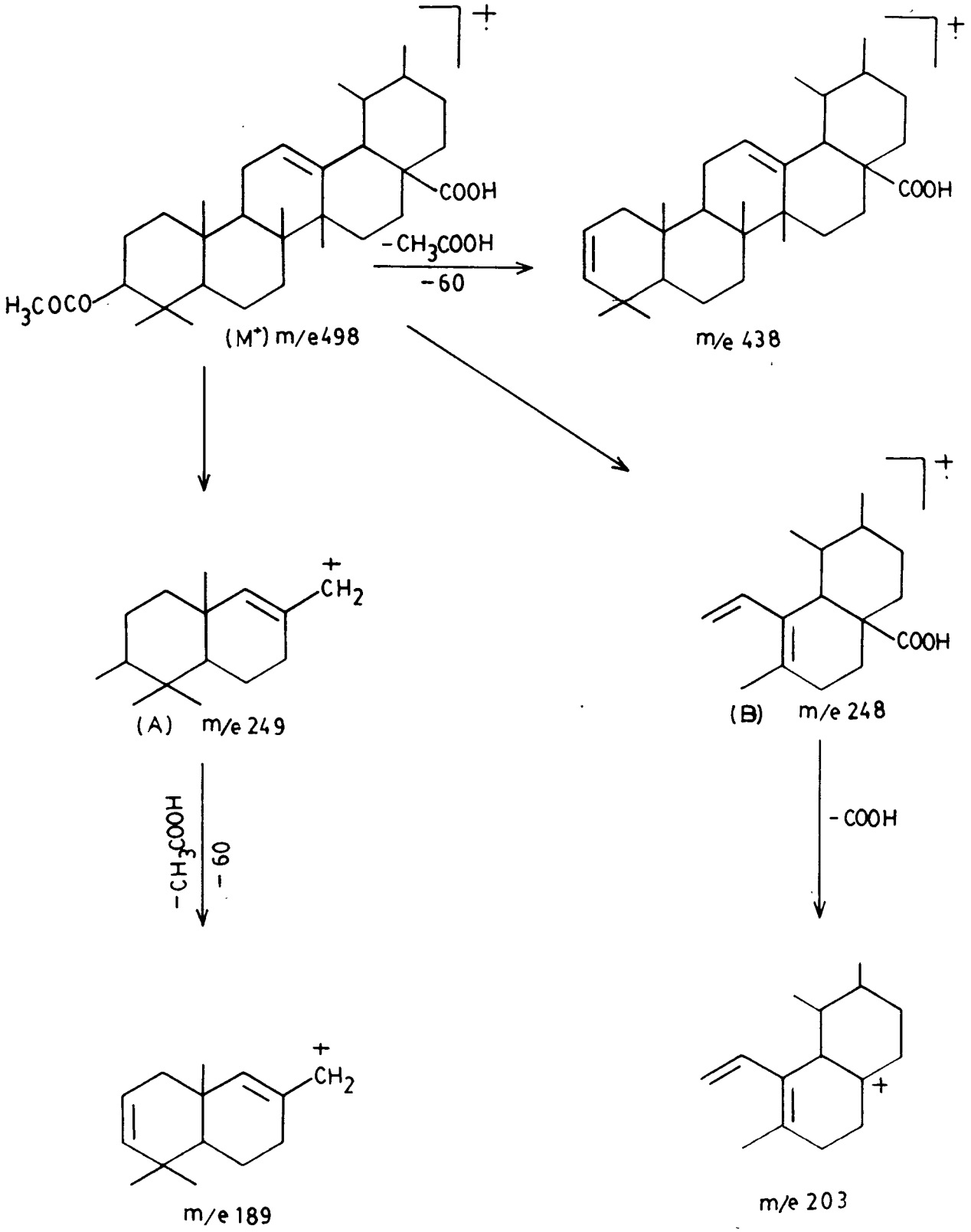

COOH
H_3COCO
$-CH_3COOH$
-60
(M^+) m/e498
m/e 438
CH_2
(A) m/e 249
(B) m/e 248
$-CH_3COOH$
-60
-COOH
m/e 189
m/e 203

CHART :- C-1

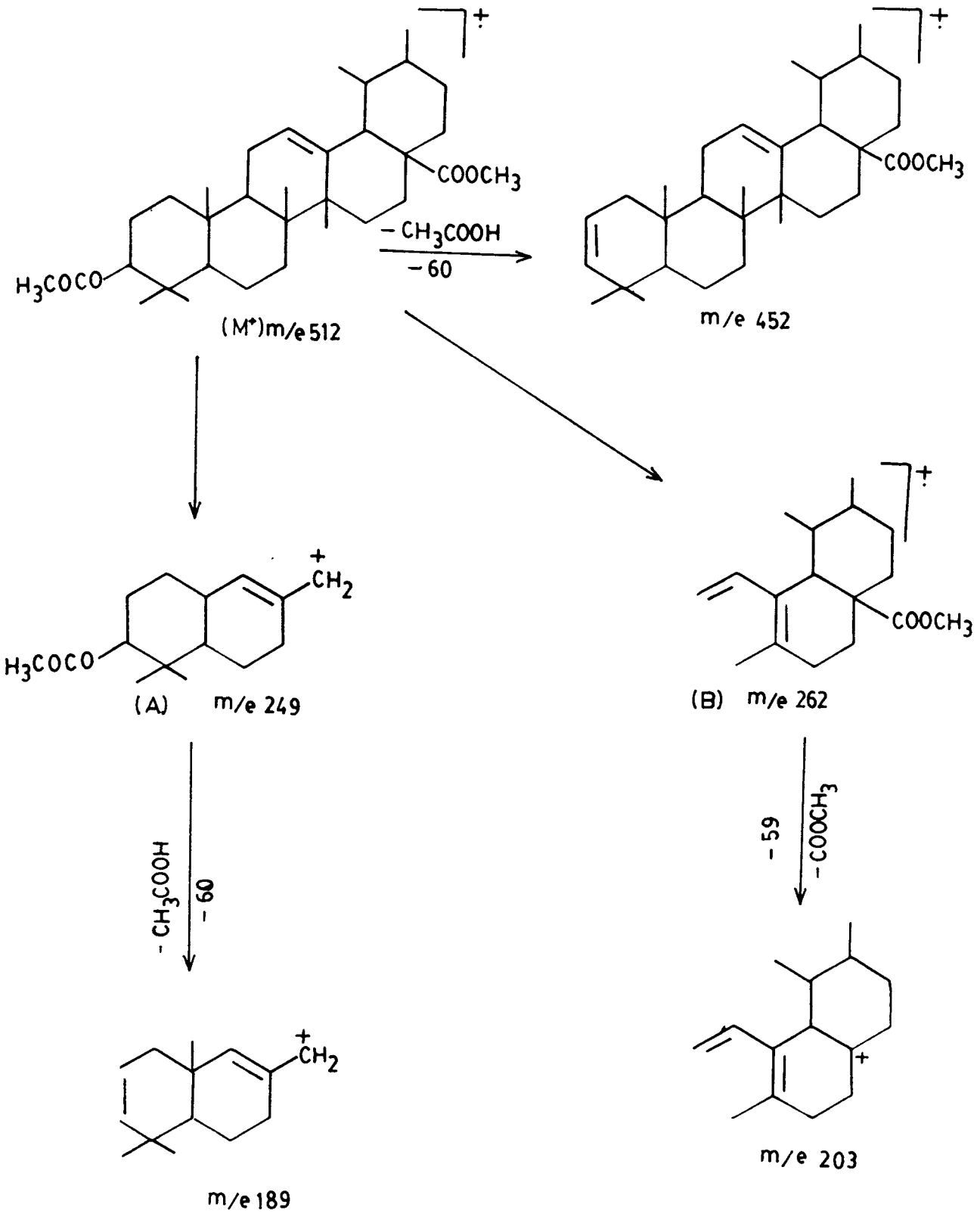

COOCH3
H3COCO
- CH3COOH
- 60
(M+)m/e 512
m/e 452
CH2
(A) m/e 249
(B) m/e 262
- CH3COOH
- 60
- 59
- COOCH3
m/e 189
m/e 203

CHART :— C–2

EXPERIMENTAL

CHEMICAL EXAMINATION OF THE FLOWERS OF ICHNOCARPUS FRUTESCENS R.Br.
(N.O. APOCYNACEAE)

The fresh flowers were collected from village Sanai in Tehsil Kiliri of District Moradabad (U.P.) and dried under shade. The dried material (400 gm) was exhaustively extracted with hot ethanol in a soxhlet extractor. The alcohol was distilled off, when a semi solid residue (50 gm) was obtained. It was extracted with petroleum ether (60-80°) eight times by refluxing on a water bath Evaporation of petrol gave a residue (12 gm).

STUDY OF THE PETROL SOLUBLE PART

The above residue obtained from petrol extract was re-dissolved in petroleum ether and left in a refrigerator overnight. A green solid (1.0 gm) separated out which was filtered and washed with petroleum ether (60-80°). It gave a red colour on Liebermann-Burchard test. Inspite of repeated attempts it could not be crystallised from different solvents. On TLC examination in toluene, ethyl acetate (7: 3) it showed two spots (one major and one minor).

Separation of Acid and Neutral compounds

The crude compound (1 gm) was heated with alcoholic caustic potash (20 gm) KOH in 300 ml of alcohol) for half an hour and then half of the solvent was distilled off. The solution was then diluted with water (2 litre) and extracted three times with ether. The ethereal extracts were combined and washed free of the alkali. It was dried over sodium sulphate (anhydrous) and removal of the ether left a neutral substance in the flask in a very small quantity.

The alkaline solution was acidified with hydrochloric acid when it gave a precipitate. This was filtered washed free of acid and dried.

ACETYALATION : The acidic compound (500 mg) obtained as above was acetylated by dissolving in pyridine (2 ml) and adding acetic anhydride (1.5 ml) and heating the contents on a boiling water bath for two hours. The reaction mixture was then left overnight at room temperature and poured next day dropwise into ice cold water when a precipitate separated out, which was filtered, washed with water and dried. It was dissolved in methanol and treated with activated animal charcoal to give a clear solution which on concentraction to a small volume gave colourless needles m.p. 254-56°.

SPECTRAL DATA

^{1}H NMR ($CDCl_3$) (δ)

0.7 - 1.05 (21 H, 7 x CH_3) 2.0 (s, 3H, $OCOCH_3$), 4.5 (t, 1H, H-3), 5.3 (m, 1H, H-12).

Mass (m/e)

498 (M^+), 438, 249, 248, 203, 189

DEACETYLATION : The acetate (250 mg) was refluxed for 2 hours with 15 ml of 5% methanolic potassium hydroxide. The solution was diluted with 200 ml water and left overnight at room temperature. It did not yield any crystalline potassium salt. The solution was acidified with hydrochloric acid and the precipitate formed was washed free of the acid and crystallised from methanol, m.p. 285-90°. It gave a red colour on L.B. test. By running its TLC in petroleum ether, ethyl formate, formic acid (93:7:0.7) alongside with an authentic

sample of ursolic acid and oleanolic acid, it was found to be identical with ursolic acid (Rf 0.19) m.mp. 286-87°.

SELENIUM DIOXIDE OXIDATION

Acetate (100 mg) in 15 cc acetic acid was heated under reflux with 100 mg freshly sublimed selenium dioxide for 2 hrs. It was poured into water and extracted with ether. The ethereal concentrate could not be crystallised. It did not show the characteristic absorption in U.V. spectrum for the members of the B-amyrin group of triterpenes.

ACETYL METHYL ESTER

The acetate (70 mg) was methylated with an excess of ethereal solution of diazomethane and the reaction mixture after leaving at room temperature overnight was evaporated to dryness. The residue on crystallisation from methanol gave a colourless crystalline compound (50 mg, m.p. 242°)

^{1}H NMR ($CDCl_3$) (δ)

0.7 -1.02 (21H, 7x CH_3), 2.0 (s, 3H, $OCOCH_3$), 3.55 (s, 3H, $COOCH_3$), 4.5 (m, 1H, H-3), 5.25(m, 1H, H-12)

Mass (m/e)

512(M^+), 452, 262, 249, 203, 189.

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CHAPTER- II

DISCUSSION

STUDY OF THE WHOLE PLANT OF CORCHORUS ACUTANGULUS LINN. SYN. C. AESTUANS (N. O. TILIACEAE).

C. acutangulus, commonly known as "Titapat" is a much branched hairy herb, upto 90 cm high. Leaves 6-9 x 3-5 cm, ovate oblong or ovate lanceolate, acute, serrate, basal serratures on each side prolonged into filiform appendages, glabrous or sparsely hairy ; petiole slinder, 1.2-2.5 cm, stipules subulate, leaves are arranged in alternate order. Flowers in axillary clusters, 1-4, small; buds obovoid. Sepals 5,oblong, apiculate. Petals 5, obovate, yellow. Stamens 10, rarely more, inserted on a short torus. Fruit -a capsule, slinder, glabrous, sub-cylindrical, 3-valved, black, ending in 3 spreading points ; seeds black, truncate.

LITERATURE SURVEY OF THE PAST WORK

The seeds of C. acutangulus are reported to contain helveticoside, corchoroside A, strophanthidine, strophanthidol, digitoxose, boivinose, 2,6-di-deoxy monomethoxy sugar and an unidentified glycoside-A (m.p. 155-60°). The sugar of glycoside-A has been identified as digitoxose.(1)

The four new triterpenic glycosides coded as corchorusins A,B,C & D isolated from the aerial parts of C. acutangulus have been characterised as longispinogenin 3-O-β-D-galactopyranoside, saikogenin F-3-O-βD-galactopyranoside, 23-hydroxy-longispinogenin-3-O-βD-galactopyranoside and saikogenin-F-3-O-βD-glucopyranosyl (1 → 2) -β-D-Galactopyranoside.(2)

A flavonol, quercetin was isolated from the fresh whole plant of C. acutangulus(3).

PRESENT WORK

The fresh plant material was collected from Hamdard Nagar Campus New Delhi. After chopping into small pieces, it was exhaustively extracted by refluxing with ethanol. The ethanolic concentrate was extracted with petroleum ether inorder to remove green colouring matter and waxy products. The petrol insoluble part was dissolved in water and filtered, further studies on the filtrate are in progress. The water insoluble material was extracted by refluxing successively with petroleum ether, benzene and methanol. The petrol and benzene extracts were found to be complex mixtures and hence studies on these fraction were not carried out further. The methanolic extract on evaporating to dryness gave a residue which gave a positive test for triterpenes. It could not be crystallised and was found to be a mixture. For the purpose of purification it was refluxed with methanolic KOH and added into water. The soluble potassium salt on acidification with HCl gave a brown precipitate which was filtered, washed with water and dried. Formation of potassium salt indicated the acidic nature of the compound. It was converted into acetyl derivative which was exhaustively extracted with benzene. The benzene solution was concentrated to a small volume and left at room temperature to give a colourless crystalline compound m.p. 265°.

CHARACTERISATION OF THE ACETATE M.P. 265°

The NMR spectrum of the compound showed signals for 6 methyl groups from δ.691 to 1.27 . The two singlets at δ 1.9 and δ 2.01 accounted for two acetyl functions. There was a triplet centred at δ 5.25 characteristic of an olefinic proton in the Δ^{12}

triterpenes, a doublet at δ4.8 for H-3 proton α to acetoxyl and a multiplet at δ 5.7 for H-2 proton α to the other acetoxyl. These data suggested that the compound may be a diacetyl derivative of some triterpene belonging either to α-amyrin or β-amyrin group. However, a comparison of the physical properties (m.p., m.mp. and co-TLC) of this compound with the diacetyl derivative of 2α, 3β, 20β-trihydroxy-urs-Δ^{12}-ene, 24, 28-dioic acid isolated recently in our labs. from another species namely Corchorus depressus confirmed its identity. A final proof was obtained by comparing the IR. spectra which were superimposable.

E X P E R I M E N T A L

STUDY OF THE WHOLE PLANT OF CORCHORUS ACUTANGULUS LINN.
SYN. C. AESTUANS (N.C. TILIACEAE)

EXTRACTION

The fresh plant material (1.0 kg) consisting of whole plant was collected from the campus of Jamia Hamdard, Hamdard - Nagar during the months of July and August and dried under shade. The authenticity of the material was established by the Department of Botany, University of Delhi,Delhi. It was chopped into small pieces and exhaustively extracted with boiling ethanol. The solvent was recovered under reduced pressure and the residue (70 gm) was extracted with petroleum ether. The insoluble mass left after petrol ex-raction was taken up in water in orders to separate into water soluble and water insoluble fractions. The water insoluble fraction (20 gm) was refluxed with petroleum ether, benzene and methanol successively. The petrol and benzene soluble fractions could not be resolved into pure components and hence further studies were not carried out. The methanolic extract gave positive test for triterpenes.

STUDY OF THE METHANOL SOLUBLE FRACTION

The residue (5.0 gm) left after evaporation of methanol could not be crystallised and hence for the purpose of purification, it was refluxed for 20 minutes with 5% methanolic KOH (50 ml). The contents were then poured into water. No insoluble material separated out. On acidification with HCl, it gave a brown precipitate (2.0 gm) which was filtered, washed with water and dried.

ACETYLATION

The above brown precipitate (2.0 gm) was dissolved in dry pyridine (5ml) and acetic anhydride (5ml) was added to it. The contents were heated on a boiling water bath for four hours and left over night at room temperature. The reaction mixture was then poured into ice cold water with constant stirring a brown precipitate separated out, which was filtered, washed with water and dried. Inspite of repeated attempts it could not be obtained in crystalline form and hence the following procedure for its purification was adopted.

PURIFICATION OF THE ABOVE ACETATE TO GIVE COMPOUND m.p. 265°

The methanolic solution of the crude acetate was evaporated to dryness and the residue exhaustively extracted with benzene. The benzene solution was concentrated to a small volume and left at room temperature to give a colourless crystalline compound m.p. 265°. It gave a red colour on L.B. It did not show any depression in m.p. (m.m.p 264°) on mixed melting with an authentic sample and had identical Rf values on T.L.C. examination in T:B:F: (5:4:1) solvent system.

SPECTRAL DATA

^{1}HNMR ($CDCl_3$) ACETATE (δ)

.691 -1.27 (6 x CH_3), 1.9 (s, 3H, $COCH_3$) , 2.01(s,3H,$COCH_3$), 4.8 (d, H-3), 5.25 (t, H-12), 5.7 (m, H-2).

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